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VELOCITY OF LONGITUDINAL VIBRATION IN SOLID RODS (ULTRASONIC METHOD) WITH SPECIAL REFERENCE TO THE ELASTICITY OF ICE[1]

BY R. W. BOYLE[2] AND D. O. SPROULE[3]

Abstract

An experimental research showing how corrections in the value of velocity of phase propagation may be made to take into account lateral inertia, and how the law (Rayleigh's) will break down at higher frequencies because of other types of vibration intervening. When the ratio of radius to length, multiplied by the mode of vibration $\left(\frac{kr}{l}\right)$ exceeds a certain figure (in the case of duralumin, 0.55) the law breaks down, and it appears that radial longitudinal vibrations intervene. From the research a simple method emerges to determine Poisson's ratio, which in the case of duralumin is found to be 0.35.

An application of this ultrasonic method is made to determine Young's modulus for ice, in order to find more consistent values than those generally quoted. The value of this modulus for ice at about 0° C. is found to be about 9×10^{10} dynes/cm^2. corresponding to a velocity of sound in it of 3.15×10^5 cm. per sec.

Most measurements of the velocity of sound in a limited medium depend ultimately on interference phenomena in a reflected wave train. In the case of vibrating rods and bars the oscillation is generally adjusted to a condition of resonance and from the measurements of one or more half-wave-lengths, the velocity of phase propagation in the rod is deduced. For the natural modes of vibration (resonance) of a free rod, the length of the rod is equal to an integral number of half-wave-lengths, or $l = k\lambda$, where l is the length of the rod, λ is the wave-length, and $k = 1, 2, 3$, etc. corresponding to the mode of vibration. From the simple wave relation $v = n\lambda$, where v is the velocity and n the frequency, it follows that $v = \frac{2nl}{k}$.

The dimensions of a rod have a considerable influence on the half-wave-length in the rod for a given imposed frequency. In the case of a cylindrical rod, if the wave-length is large compared with the radius of the rod, or, what amounts to the same thing, if the ratio of the radius to the length, $\frac{r}{l}$, is

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Contribution from National Research Laboratories, Ottawa, Canada. This paper was read before Section III of the Royal Society of Canada in May, 1931.

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small, it is generally taken that $v = \frac{2nl}{k} = \sqrt{\frac{E}{\rho}}$, where E is Young's modulus of tensile elasticity and ρ is the density of the material. This is not strictly accurate, for the occurrence of Young's modulus connotes that the sides of the rod are not prevented from lateral bulging and shrinking, and bulging and shrinking involve a use of energy not accounted for in the formula. The value of the modulus employed should be the adiabatical or dynamic value and not the isothermal, which is generally determined by static or extensometer methods. In the case of solids, however, the difference between these two moduli is very small and is usually neglected.

For the gas column in a Kundt's tube $\frac{r}{l}$ generally has values of 0.005 to 0.1. For solid rods the simple wave relation above is often used with no particular regard to the important $\frac{r}{l}$ ratio. In the case of solids, for $\frac{r}{l} = \infty$, *i.e.*, for an infinite solid medium, in which the lateral bulging and shrinking which occurs in a rod is no longer possible, the velocity (V_0) is given by $V_0^2 = \frac{(1-\sigma)}{(1+\sigma)(1-2\sigma)} \frac{E}{\rho}$, where σ is Poisson's ratio.

It is impossible to perform sound experiments on "an infinite solid"—but a method to determine the velocity appropriate to the case emerges from the work of Boyle, Lehmann and Froman (2, 3). They found, by working with ultrasonic waves in water, incident perpendicularly on a partition of homogeneous material of thickness t, that the reflection was a maximum for t = an *integral odd number* of *quarter*-wave-lengths, and transmission a maximum for t = any *integral number* of *half*-wave-lengths. In some of the experiments the "partition" was merely a thin disk constituting the vane of a torsion pendulum by means of which the measurements were taken, in which case the vane itself was made to indicate the ratio of reflected or transmitted to incident energy. By plotting either the transmission or reflection ratio on the thickness, at a constant frequency of imposed waves, the wave-length in the material of the disk was found; the product of this wave-length with the frequency gives the velocity. Such velocities are always considerably greater than that computed for the material from quoted values of density and Young's modulus as determined by extensometer methods. For example, in duralumin, the velocity by the torsion pendulum ultrasonic method was found to be 6.48×10^5 cm. per sec., while from extensometer methods it was computed to be 5.16×10^5 cm. per sec. Such large divergences could not be explained by attributing them to experimental errors, and it is evident that assumptions made in applying the simple relation $V = \sqrt{\frac{E}{\rho}}$ are not satisfied in the conditions of the ultrasonic experiment. In the extensometer method $\frac{r}{l}$ is small, generally much less than 0.1, but in the ultrasonic method formerly described $\frac{r}{l}$ is relatively large, generally greater than 5. For example, in the case of the duralumin, Poisson's ratio $\sigma = 0.35$, the density $\rho = 2.79$, and $E = 7.5 \times 10^{11}$;

using the relation for an infinite solid, *viz*, $V_o^2 = \frac{(1-\sigma)}{(1+\sigma)(1-2\sigma)} \frac{E}{\rho}$ (1), it follows that $V_o = 6.57 \times 10^5$. This is in good agreement with the value 6.48×10^5 as determined with thin circular disks by the ultrasonic method. Thus it is indicated that with these thin disks the ratio $\frac{r}{l}$ is large enough to make the above formula approximately applicable, and that the velocity derived by this ultrasonic pendulum method is about the same as that deduced if the "elongational elasticity" (j) instead of Young's modulus is used in the simpler relation, $V_o = \sqrt{\frac{j}{\rho}}$ (1, pp. 125, 190).

Effect of Lateral Inertia

Early theoretical work on elastic vibration of a free cylindrical rod by Pochhammer (21) and Chree (7) was followed by that of Love (13, p. 289) and Rayleigh (23, pp. 251-253). Rayleigh's solution for longitudinal vibration was simple and practical and made a correction for the effect on the period of vibration of lateral inertia. The correction however took no account of any damping of vibration by solid viscosity or of the possible occurrence of resonant vibrations other than longitudinal lengthwise (such, for example, as radial longitudinal or torsional,) and it is on this account that the corrections will be incorrect after certain limits of frequency have been exceeded. The purpose of the present investigation was to demonstrate experimentally how these limits might be found.

The lowering of phase velocity on account of lateral inertia in a thick cylindrical rod may be deduced from the relation given by Rayleigh, as

$$V_2 = \frac{V_1}{1 + \frac{k^2\sigma^2\pi^2 r^2}{4l^2}},$$

where V_1 is the velocity for a thin rod, *i.e.*, where $V_1 = \sqrt{\frac{E}{\rho}}$ may be safely applied, and V_2 is the velocity in a rod of significant radius (r) as compared with the length (l); k is the integer, 1, 2, 3, etc., representing the particular mode of vibration.

For a fixed length of rod, if V_2 were constant for all longitudinal resonances the frequencies of successive modes would be in the ratio 1, 2, 3, etc., that is, the overtones series would be harmonic. But a change in V_2 with successive modes, as is indicated by Rayleigh's expression, results in departure from the harmonic series. The phase velocity V_2 according to this expression is a function of $(\frac{kr}{l})$, *i.e.*, depends on the radius and length of the rod, and on the mode of vibration.

Rayleigh mentioned the improbability of confirming experimentally his correction for lateral inertia because of the difficulty of experiment then existing with rods short enough and thick enough to make the correction appreciable; but since the advent of ultrasonic oscillators, operated by adjustable frequency oscillating electrical circuits, this difficulty disappears. During the Great

War experimenters in England investigated to some extent the longitudinal oscillations in steel rods excited electromagnetically in the phenomenon of magneto-striction, but no results were published. More recently, Quimby (22) and also the present writers (5) experimented with metal rods excited into oscillation by the piezo-electric effect of quartz. Pierce (20) and also Muzzey (17) determined elastic constants and velocities with high precision in rods or tubes of certain ferromagnetic metals by Pierce's high frequency magneto-striction oscillators. By this method, Muzzey confirmed Rayleigh's formula for small values of $\frac{kr}{l}$ in the case of stainless steel. By a very interesting method of substitution Klein and Hershberger (10) measured the longitudinal velocity in a slab of solid with their "supersonic interferometer". Setting up in the interferometer a stationary wave system in a column of liquid in which the velocity is known, by inserting a parallel faced slab of solid in the liquid path, the nodal planes are displaced and the displacement measured. The difference in *acoustical path* with the solid absent and then present could thereby be determined and the velocity in the solid deduced.

The present work by the authors was carried out four years ago, and though the determinations claim no high degree of precision it was thought advisable to put them on record. The paper is an amplification of results and indication of the method which already have been briefly communicated (5).

Experimental

Cylindrical metal rods, *A*, of uniform diameter and equal length were cemented to each side of a quartz disk, *B*, so that the rods were coaxial, as is shown in Fig. 1. Terminals from a valve oscillator were connected to the metal rods. With this arrangement the even numbered modes of vibration were quite weak, and the numerical results here shown were obtained from the odd numbered modes only.

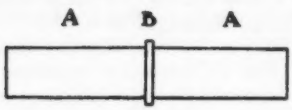


FIG. 1.

Under the above conditions the oscillating electric field imposed on the quartz sets up by piezo-electric action a pressure wave which travels out into the metal rods. When the frequency of the electric field corresponds to the natural frequency of one of the modes of mechanical vibration of the quartz-metal combination, the intensity of the mechanical vibration is increased greatly due to resonance. This increase may be detected by any one of several methods as will be indicated later. In no experimental case was the wave-length in the solid rod so short that a sharp ultrasonic "beam" could be emitted from the quartz; for such a condition the diameter of the rod would have to be many times a wave-length. The reverse was the case.

Other arrangements than the one described were used to start the vibrations. Various combinations of long and short rods using active quartz were possible, even to placing a piece of quartz at one end only. The method of placing a strip of quartz on the side of a rod gave comparatively feeble oscillations. In addition iron rods were made to vibrate by the direct magneto-strictive action of the alternating fields of magnetic force near the oscillating coils.

The chief objection to using a composite rod composed of a quartz layer and lengths of another material is that an appreciable correction may become necessary if the velocity of sound in the quartz is not the same as the velocity of sound in the experimental material. The correction may be found as follows: The necessary condition for resonance at the fundamental frequency is that the sound travels the whole length in half a period. Let l = length of rod, d = thickness of quartz, V_q = velocity of sound in quartz, V_m = velocity of sound in the metal pieces, n = frequency and T = period $\frac{1}{n}$. The time required for sound to travel through the metal is $\frac{l-d}{V_m}$ and the time required for the sound to travel through the quartz is $\frac{d}{V_q}$. Hence $\frac{l-d}{V_m}+\frac{d}{V_q}=\frac{1}{2}T=\frac{1}{2n}$, whence $V_m=\frac{(l-d)\,2nV_q}{V_q-2nd}$. Taking $V_q=5.5+10^5$, and $d=0.2$ cm., it was found that the calculated correction for the rods used was in all cases less than 1%, and therefore not more than the variation in velocity from sample to sample of material used for the rods. Hence its application was unnecessary within the limit of accuracy permitted by these experiments.

The method of experiment was to set the oscillator vibrating, carefully adjust the electrical frequency until a resonant longitudinal mechanical vibration of the oscillator could be detected, then measure the electrical frequency with a calibrated Hertzian wave meter. The method suitable for the detection of the resonant longitudinal vibration depended somewhat on the power and frequencies employed in the experiment. The following devices were successfully employed at various times, the particular one selected depending on the circumstances of the work. Usually the observed oscillations were generated by a 250-watt valve oscillator set. Experiments of the same kind as are described here have been generally carried out by other workers at much weaker energies.

(1) By imposing rectified 60 cycles per sec., or higher periodicity, alternating potential on the plate of the valve oscillator a "tonic-train" oscillation was made to modulate the high frequency mechanical vibrations of the rod. A stethoscope with a cup-shaped soft rubber "listening" tip could be applied to one of the vibrating ends of the rod to detect the "tonic-train" when the rod vibrated at or near its resonant frequencies. A stethoscope with double tip, as shown in Fig. 2 which is self-explanatory, was devised to utilize in detection the sound energy from both ends of the oscillating rod. The same results were obtained using stethoscopes with either a single or double tip.

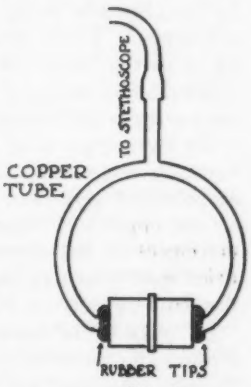


FIG. 2.

(2) By placing one end of the vibrating rod in a suitable liquid, cavitation (bubbling) of the liquid could be produced at some of the resonant frequencies of the rod (6). Bubbles of dissolved gas could be

driven even from ordinary water, thus indicating that in some cases the energy intensity emitted from these small resonators could sometimes exceed the intensity in beams from much larger transmitters.

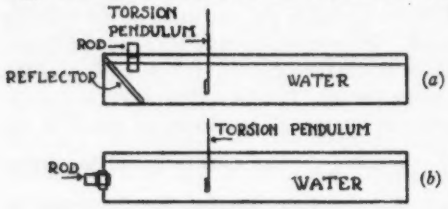


FIG. 3.

(3) As indicated in Fig. 3, (*a* and *b*), the ultrasonic beam emitted from the end of the rod at resonance could be used to detect some of the resonant frequencies, the indicator being the deflection of a torsion pendulum suspended in a suitable liquid in a small tank.

(4) By the use as detector of an ordinary coupled low power radio receiving set, in a variety of ways such as follows— (a) The simple procedure often used in experiments with piezo-electrically controlled radio oscillators—as the frequency of the rod oscillator is adjusted to pass through a longitudinal resonance, a sharp "chirp" is heard in the telephones of the coupled radio circuit. This useful phenomenon is due to the fact that the oscillating rod takes extra energy from its driving circuit when the frequency (f_1) of that circuit is the resonant frequency of the rod; but on slightly changing the frequency of the driving circuit to f_2, the rod feeds the energy back into the circuit at the frequency f_1, so producing an audible beat or "chirp" in the phones for values of f_2 near f_1. (b) The fact that the oscillating rod takes extra energy from its driving circuit at the frequency of resonance may be shown by a "kick" of a galvanometer of requisite sensibility placed in the oscillator plate circuit, as indicated in Fig. 4. (c) The rods made to vibrate by mechanical instead of electrical means by tapping them sharply at one end with a hammer. This method can be used only up to certain limits of frequency (12). A coupled radio receiving set can detect the resonances in (4a) above.

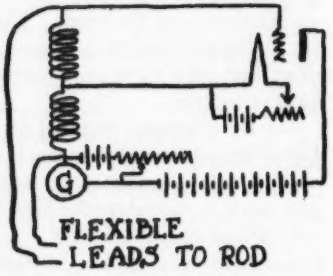


FIG. 4.

(5) By supporting the oscillating rod vertically and placing light particles, such as powdered cinders or small glass beads, on the upper end, resonant frequencies can be detected by the shifting and movement of the particles at these frequencies. The motions of these particles were found to yield an insight into the possible complex vibrations in the rod, especially at the higher frequencies, as discussed later (Fig. 8).

(6) Some of the experiments were carried out when the rod was oscillating in the audio-frequency range, in which cases the simplest method of detecting resonance was to adjust the frequency until the maximum audibility of emitted sound was heard. Some of the other methods enumerated above could also be used in this case.

Whatever the method employed to detect the resonances, great care must be exercised by checking and re-checking between the methods to be certain that it is a *longitudinal resonance* and not resonance of another type of vibration which is observed, otherwise the calculations for velocity have no validity. This important precaution may easily be overlooked.

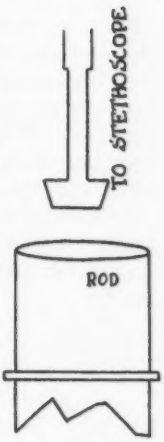
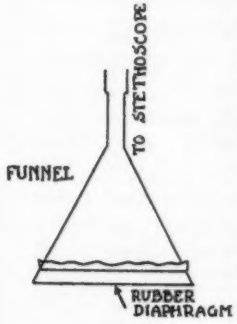


FIG. 5.

[Note: In working with detection method described above, in many cases the note of the tonic train becomes inaudible or nearly so when the listening tip of the stethoscope is lifted barely clear of the vibrating surface (Fig. 5) but becomes audible at a point further away. Calculation revealed the fact that the distance from the listener to the end of the oscillating rod was half a wave-length of the emitted ultrasonic waves in the air. By employing rods of greater than the usual diameter, and increasing the effective reflecting surface of the listener, it was found possible in some cases to detect nodes and antinodes in the air for as many as 100 successive half-wave-lengths Knowing the value of the velocity of sound in air it was possible to determine the frequency of the oscillator by this method; or, on taking the Hertzian wave-meter frequencies as standard, a check of better than 1% in the velocity of sound in air was easily obtained this way. The simplest method of obtaining a large reflecting surface for the listener was to stretch a very thin rubber diaphragm over the mouth of a thistle funnel as shown in Fig. 6. This experiment is of the same type as that described by Pierce (19) and by Hubbard and Loomis (9), who determined the nodal positions of the reflector by the reaction of the reflected ultrasonic wave on the electrical circuit. In the present case the nodal positions were determined by the reactions of the wave on the reflector itself, through the audibility of the tonic train.]



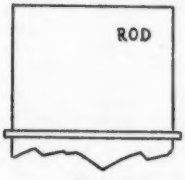


FIG. 6.

Results

The first rod used was designed so that the correction for lateral inertia would not be considerable for its first (or fundamental) mode of vibration, but would be appreciable for the higher modes. Duralumin was selected as a suitable material. Two cylinders of duralumin 7.0 cm. long and 5.1 cm. in diameter were turned on the lathe. These were cemented coaxially to the opposite faces of a piezo-electric quartz disk 0.19 cm. thick, thus making a composite rod 14.19 cm. long. The velocity of sound in this rod at room temperature as determined from $V = \sqrt{\frac{E}{\rho}}$, using the known values of density and Young's

modulus, *viz.* 2.79 and 7.4×10^{11} respectively, was 5.16×10^{5} cm. per sec.; the velocity in this rod determined experimentally by the ultrasonic methods here described was 5.08 cm. per sec.

A long series of experiments employing many rods of different proportions was now carried out. The rods were all carefully machined from the same large piece of duralumin, most of them being shortened lengths of the longer rods and smaller diameter pieces of the thicker rods. The temperatures were always around the ordinary room temperature of 18° C.

In some of the rods the diminution of velocity by lateral inertia was appreciable even for the first mode of vibration, in others the diminution was inappreciable up to the seventh mode. In Table I are assembled the numerical results.

TABLE I

EFFECT OF DIMENSIONAL CHANGES ON VELOCITY

r	l	k	kr/l	Velocity, cm./sec., calc. from Rayleigh's formula	Frequency in cycles/sec.	Velocity, cm. sec., from experiment
0.63	14.2	1	0.0444	5.10×10^{5}	17900	5.09×10^{5}
		3	0.133	5.08	53500	5.07
		5	0.222	5.03	88300	5.01
0.95	14.2	1	0.0668	5.10	17900	5.09
		3	0.220	5.04	53200	5.04
		5	0.334	4.93	88000	5.00
0.95	61.2	3	0.0466	5.10	12520	5.11
		5	0.0776	5.09	20900	5.12
		7	0.109	5.08	29200	5.11
		9	0.140	5.07	37350	5.07
		11	0.171	5.05	45500	5.06
		13	0.202	5.04	53600	5.05
1.25	6.21	1	0.201	5.04	40600	5.05
1.25	12.2	1	0.103	5.08	20700	5.05
		3	0.307	4.96	61000	4.96
		5	0.513	4.72	94000	4.59
1.25	14.2	1	0.0880	5.08	17900	5.08
		3	0.264	4.99	52700	4.99
		5	0.440	4.81	83800	4.76
2.55	8.25	1	0.309	4.96	30000	4.95
2.55	14.2	1	0.179	5.05	17900	5.08
		3	0.539	4.68	48500	4.60
		5	0.898	4.18	61500	3.50

From the table and curve, Fig. 7, it may be seen that with duralumin for $\frac{kr}{l}<0.1$ the effect of lateral inertia is not very appreciable. In the range $\frac{kr}{l}>0.1$ and <0.55 Rayleigh's expression gives the velocity closely enough for most purposes. For $\frac{kr}{l}>0.55$ the experiments demonstrated that the

modes of vibration are more complex, and that it is virtually impossible to obtain trustworthy observations in the experiments.

A study of the behavior of dust particles on the vibrating end surface of the rod oscillator throws additional light on what is taking place. Dust of sifted cinders or duralumin filings proved suitable for this use. For the modes of vibration giving numerical results consistent, as above, with Rayleigh's correction, the dust particles were simply agitated up and down on the end of the rod without showing any tendency to arrange themselves in any particular pattern on the vibrating end surface; this might be expected for a pure longitudinal oscillation. But for vibrations when $\frac{kr}{l} > 0.5$ a distinctly different type of behavior was observed. At certain frequencies, for example between 84000 and 300,000 cycles/sec. for a rod 48 cm. long and 5.1 cm. diameter, the dust arranged itself in various patterns. Four-, six-, eight- and twelve-pointed stars could be obtained. (The photograph shown in Fig. 8 represents an eight-pointed star.) Some sort of radial symmetry must be assumed to account for such figures of radial symmetry.

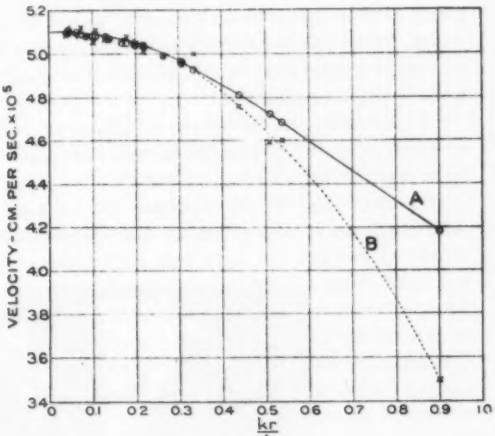


FIG. 7.

Another type of behavior of the particles was even more striking. At certain frequencies the particles were observed to move continuously in a circle about the centre of the rod. For others, the particles near the outer edge of the end of the oscillator moved in a clockwise direction, while those near the centre moved in the counter direction. At times, little whirls formed off to one side of the centre.

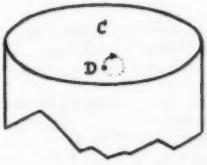


FIG. 9.

A combined torsional and longitudinal vibration might account for these circular motions, if the two forms of vibration had a fixed phase relation. For example, consider a small element, D, of the surface at one side of the centre, C, Fig. 9. If the rod is executing combined torsional and longitudinal vibrations in an appropriate phase relation, then at a given instant the point D may be moving up due to the longitudinal vibration and, at the same time, beginning to move to the right due to the torsional vibration. A quarter period later it would be beginning to move down due to the longitudinal vibration and would be moving to

the right due to the torsional vibration. It can be seen that in such a case the particle D would be shot to the right once every cycle. If all parts of the surface equidistant from the centre were vibrating in the same manner, every surface particle on this ring would be shifted in the same manner and would behave as the particles are observed to do. Harmonics of the torsional vibration would explain the existence of more than one circle of particles as were sometimes observed.

The intricate complexities of vibration, which may exist at high radio-frequencies in the quartz blocks and rods used in radio frequency stabilization have recently been demonstrated by similar dust figure methods to the above.

It is clear that the experiments warn against any idea that a thick rod will always vibrate in a longitudinal manner because it is supplied with longitudinal vibrating energy. It is certain that types other than longitudinal vibration will take place. Even in thin rods more than one type of vibration may exist, though this is not readily observable; but as the rod thickens the distribution of the energy into more than one dominant type may, by their resonances, become more easily observable. In reality a rod, especially a thick one, when supplied with any one type of vibrational energy may become a vibrating system of almost all other types.

Incidental

(1) *Audible Beat Notes*

During the above experiments an interesting fact was sometimes observed. An audible note could be emitted from the thick rods when they came into resonance, even when the driving electrical oscillations were at the ultra-audible frequency of 100,000 cycles per sec. or more. This is readily explained by the fact that in the successive modes of vibration of a rod, beats may be caused between two or more of the overtones. If the beat frequency is lower than about 20000 cycles per second, the beat note will be audible, provided the intensity is sufficient. This was often the case.

(2) *Determination of Young's Modulus for Metals*

As an application of the methods described here, determinations were also made of the velocities in rods of iron, brass, magnesium, and type metal. Results for these rods are compared with those computed from values of Young's modulus and density and are shown in Table II.

TABLE II

COMPARISON OF VELOCITIES OF LONGITUDINAL VIBRATION IN METAL RODS OBTAINED BY THE ULTRASONIC METHOD WITH THOSE COMPUTED FROM VALUES OF YOUNG'S MODULUS

Material	Velocity by ultrasonic method, cm. per sec.	Velocity from $V=\sqrt{\frac{E}{\rho}}$, cm. per sec.	Material	Velocity by ultrasonic method, cm. per sec.	Velocity from $V=\sqrt{\frac{E}{\rho}}$, cm. per sec.
Iron	4.95×10^5	4.99×10^5	Magnesium	5.02×10^5	4.98×10^5
Brass	3.47×10^5	3.50×10^5	Type metal	1.71×10^5	1.72×10^5

FIG. 8

(3) *Determination of Compressional Elasticity of Non-metallic Solids*

As a further application the compressional elasticity of a non-metallic solid, *e.g.*, marble, was determined.

Two rectangular prisms of marble were sealed to tin-foil electrodes which in turn were sealed to opposite faces of the quartz—to compose an ultrasonic oscillator of marble. Fig. 10 represents the arrangement. The marble prisms were 8 cm. long with sections 2 by 4 cm. Velocity so measured was 4.06×10^5 cm. per sec. A quoted value gives the velocity 3.8×10^5; it is likely that the determination by the ultrasonic method is the more correct.

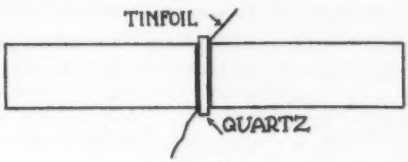


FIG. 10.

Discussion

(a) The longitudinal velocity in a "thin" cylindrical metal rod is given by $V_1=\sqrt{\frac{E}{\rho}}$, if a "thin" rod is defined as one for which Rayleigh's correction for lateral inertia is inappreciable. Young's modulus as determined by longitudinal vibrations in such a rod will be, within the experimental error, the same as the modulus obtained by static loading, although the stresses set up in these two cases are so different Researches of Swift (24) and of Pichot (18) have already shown this to be the case for flexural vibrations in rods of metal and stone This agreement might be expected as the stresses in the two cases are identical in type, both taking place in the direction of the long axis of the rod. However, a discussion of possible differences between the dynamic and static methods in other cases is included later in the paper in the section concerning the work on ice.

(b) The formula for the velocity of sound in an infinite solid may be written $V_o^2=\frac{(1-\sigma)}{(1-\sigma)\,(1-2\sigma)}\cdot V_1^2$. V_o may be measured by the method employed by Boyle and Lehmann (2), while V_1 may be determined by the method described here. Hence, σ is determined. This result is of special interest as Poisson's ratio is a most difficult constant to determine accurately by direct experiment. For duralumin, by the method here, σ is computed to be 0.35.

(c) Also, σ may be obtained from the expression for (V_2), since $\sigma=\frac{(V_1-V_2)4l^2}{k^2\pi^2r^2V_2}$ where V_2 = vel. in thick rod, and V_1 = vel. in thin rod. In this case V_1 may be determined from the first mode of vibration and V_2 from any succeeding mode by using a rod of suitable dimensions within the range of applicability of Rayleigh's formula. This method has the advantage of permitting Poisson's ratio to be measured from simple observations on the same small piece of material.

(d) In Muzzey's experiments (17) on stainless steel by the magneto-strictive method a range of $\frac{kr}{l}$ from zero up to a value of 0.25 was covered and good agreement with Rayleigh's formula was shown. The results of the present

experiments on duralumin within the same range of $\frac{kr}{l}$ agree with this conclusion, and find that $\frac{kr}{l}$ has to reach a value of about 0.55, in the case of duralumin, before the longitudinal velocity diminishes by more than 2%. It is at higher values of $\frac{kr}{l}$ in this material that the law more seriously breaks down, for the chief reason that resonances of other types of vibration are beginning to intervene. Possibly also viscosity under these conditions is having a greater effect. Other materials will have a corresponding set of values of $\frac{kr}{l}$ which will determine the types and modes of vibration that are possible.

(e) It should be noticed that in the present experiments detection of the longitudinal resonances of the rods was made sometimes by mechanical means, such as "listening" devices, torsion pendula and cavitation, and sometimes by electrical reactive methods like telephones or galvanometers acting reactively in the electrical oscillating circuit. No differences in the resonant frequencies could be found when using mechanical or reactive detectors. When a reactive electrical detector is used certain considerations must enter into the interpretation of the value of frequency to which the detector makes its maximum response. In the case of magneto-striction it can be seen from Pierce's (20) theory of the oscillator, and as pointed out by Muzzey (17), that the equations of motion for the mechanical vibrations in the rod should include terms to represent (a) the forcing of the rod by magneto-striction and (b) the magnetic reaction of the vibrations of the rod on the magnetic induction through the coils. On solving the complete equation for the resonant frequencies (which will be the frequencies indicated by a reactive detector) the same form of relation for frequency will be found as when these extra terms were omitted, but the "constant" representing the elasticity E is now increased by a small amount, (which is the product of two coefficients, one of them mechanical, the other magnetic).

Similar considerations must apply in the piezo-electric oscillator—the extra terms in the equation of motion will represent (a) the forcing of the rod by piezo-electric action and (b) the electrical reaction of the vibration of the rod as a back e.m.f. in the driving circuit. In the formula for resonant frequency (as detected by a reactive detector) the constant E will be increased slightly. This consideration would not apply in the case of a mechanical detector outside the driving electrical circuits.

As mentioned above, in the experiments on duralumin no differences in resonant frequencies were detected when using mechanical and reactive detectors, consequently the product of the two coefficients in the piezo-electric case must be small and negligible in comparison with E. Muzzey's experiment showed that in the case of magneto-striction oscillators of stainless steel, the value of E_1 came out to be about the same value as would be expected for Young's modulus, that is, the product of the two coefficients is negligible in comparison with E. This product in the piezo-electric case is likely to be even smaller than that in the magneto-strictive.

Part II. Special Reference to the Elasticity of Ice

Young's Modulus for Ice

In association with work on iceberg detection by means of an ultrasonic beam, carried out by Boyle and Reid (4) in the Gulf of St. Lawrence, 1924, it became expedient to apply the method described here to the determination of the elastic constant of ice. Investigations to determine Young's modulus for ice had been carried out before by various workers employing static bending methods, but exceedingly discordant results have been obtained.

A glance at Table III reveals that this modulus for ice either varies a great deal from sample to sample, or else something is very uncertain in the static methods of experiment with this material. The application of the methods of this paper indicates a small variation from sample to sample of ice but nothing of the order indicated in Table III.

TABLE III

YOUNG'S MODULUS FOR ICE

Observer	Young's modulus kg. cm.^{2}	Observer	Young's modulus kg. cm.^{2}
Moseley	92700	Hess	27600
Benan	60000	Weinberg	50000
Renach	23630		

A further lack of agreement is to be found on examining the results of investigators who were searching for a difference in Young's modulus in directions parallel and perpendicular to the optic axis. Some claim no appreciable difference, others large differences of the order indicated by the results of Matsuyama (14). By the bending method Matsuyama found that Young's modulus for the bar of ice, cut so that the optic axis was perpendicular to the long axis of the bar and also to the plane of bending, was 9400 kg.cm.$^{-2}$*; a bar cut so that the optic axis was parallel to the plane of bending and also to the long axis, gave a modulus of 18900 kg.cm.$^{-2}$, while one with the optic axis parallel to the plane of bending but perpendicular to the long axis yielded a modulus of 6100 kg.cm.$^{-2}$. As will be seen later Young's modulus for ice as determined by ultrasonic longitudinal vibrations indicates that any variation of the modulus with respect to direction in the ice crystal is not great.

[Note. A more accurate determination of Young's modulus for ice is of incidental interest on another account. Thornton (25) pointed out the possibility of a simple relation existing between the thermal conductivity, Young's modulus, and the density, of solid non-metallic insulators. It appeared that the expression $K = E\rho = V^2\rho^2$, (order being omitted) where K = heat conductivity, might be valid generally. In computations from tabulated constants Thornton found that this relation held in a fair number of cases. Clarke (8) pointed out that this relation did not hold for a very homogene-

* *In the original paper there appears to be an error in the naming of the units expressing the results.*

ous insulator like an optical glass and thought that the agreement shown by the materials chosen by Thornton was apparently a matter of chance. Thornton's relation for ice could be checked if some indication could be obtained as to which of the many quoted values of Young's modulus was correct. Taking the value of E for a sample of ice at 20° C. as found in this research (p. 616) as 9×10^{10}, and ρ as 0.92, the product $E\rho$ is 8.3×10^{10}. The quoted value of heat conductivity for ice (K) is 0.051. The suggested relation $K=E\rho=V^2\rho^2$ (order being omitted) therefore does not even approximately hold.]

From the foregoing experiments of Part I on metal rods it can be seen that a simple, rapid and reasonably accurate dynamic method is available for finding Young's modulus of any solid. Two advantages in particular are associated with the method of high frequencies, namely, a small sample of the experimental material is sufficient and more convenient than a large one, and the frequency is readily measurable to considerable accuracy. Hence, it may be considered that values of the elastic constant for ice determined by this method are more dependable than those from former determinations.

In making the ultrasonic ice oscillators, owing to the fact that ice is a good electrical insulator, it was necessary to cement tinfoil to each side of the active piezo-electric quartz with sealing or other wax before freezing the ice rods to the quartz disk, as indicated in Fig. 10. The samples, rectangular prisms, were first cut roughly from the ice block with a metal saw, and then shaped more exactly by the melting of their surfaces as and where required on a slightly warmed flat metal plate. The sections of the prisms were about 2 cm. square, and smaller sections gave the same results. Since no suitable methods of artificial refrigeration were available, the experiments were performed out of doors, during the months of November, December and January, 1927-28. The rods of ice were placed outside a window of the experimental room at times when the temperature was suitable.

It cannot be said with exactness that the ice samples employed here were cut from a single ice crystal, for such are often difficult to obtain in sufficient size, and twinning is very common. The samples were cut from perfectly clear blocks of ice taken from a river where freezing had taken place slowly in relatively quiet and unagitated water. Although not invariably true, generally it is the case that when freezing takes place slowly in still water, the ice crystals form with the optic axis in or very near the vertical. Hence, in the present experiments, it was considered that the direction of the optic axis of the samples was in or near the direction of the vertical in the naturally occurring block of ice.

Effect of Temperature

The variation with temperature of Young's modulus for ice was investigated as well as possible under the local conditions from 0° to −35° C. This was done by taking observations on the exposed ice oscillators as the outside air temperatures changed as noted. Results are given in Table IV. A small increase in E with a decrease in temperature was found. Here the inability to

control the temperature was a handicap for a time lag in the value of E behind the temperature was noticed. The heat conductivity of ice is low, but if this lag had been due only to the time required for the ice to take up the temperature of the surroundings, the lag would have been greater for the larger rods. This was not the case. The effect might be worth studying in a laboratory where low temperature facilities are available.

TABLE IV

VARIATION WITH TEMPERATURE OF YOUNG'S MODULUS FOR ICE. SAMPLE WITH OPTIC AXIS PARALLEL TO THE LONG AXIS OF THE ROD

l, cm.	Temp., °C.	Frequency, cycles per sec.	Velocity, cm. per sec.	E, dynes per cm.^{2}
12.3	−9.0	12930	3.18×10^5	9.29×10^{10}
11.9	−10.0	13530	3.21×10^5	9.48×10^{10}
11.9	−30.0	14000	3.33×10^5	10.2×10^{10}
12.3	−35.0	13950	3.43×10^5	10.9×10^{10}
11.9*	At some temp. between −30° and −60° C.	14880	3.54×10^5	11.5×10^{10}

**The last result quoted in the table came from an attempt to extend the temperature range downward by placing the ice oscillator in a thermos bottle and cooling with "carbon-dioxide snow". Several readings were taken as the ice cooled. But due to the unequal contraction of the ice and quartz, the rods fell apart at some temperature between −30° and −60° C. and the experiment could not be repeated.*

A qualitative idea of the effect of temperature on the solid viscosity and damping of vibration in the ice was incidentally observed from the intensities of the emitted note when the rods oscillated at audio-frequencies. On cold days (temperature about −30° C.) the note of the resonant ice rods was plainly audible at a distance, but on relatively warm days the sound was barely audible even close to the oscillator.

The results with ice by this method cannot be as accurate as with other solids for a number of reasons. One is that the material does not lend itself to the work so readily. Other reasons will be discussed later. It was found that there were variations in E from 9.0×10^{10} to 10.5×10^{10} even with rod oscillators cut from the same block of ice and in the same direction—changes of temperature of course explaining some of these differences.

Effect of Direction in the Ice Crystal

In order to determine the possible effect of orientation of the optic axis with respect to the long axis of the rod oscillator, many rod samples were cut from the same solid block of ice. In some samples the long axis of the rod was parallel to, in others perpendicular to, and in others inclined at about 45° to, the vertical in the ice as frozen. Results are given in Table V.

A glance at Table V reveals the fact that any variation there may be in E in different directions in the ice crystal is slight in comparison with the great variation quoted from other investigations, and not greater than the variation from sample to sample of ice cut in the same direction from the same block. This is not equivalent to saying there is no variation in different directions

TABLE V

RESULTS OBTAINED WITH ICE SAMPLES CUT IN DIFFERENT DIRECTIONS

Direction of cut	l, cm.	Temp., °C.	Frequency, cycles/sec.	Velocity, cm./sec.	E, dynes/cm.^{2}
Cut parallel to the vertical	13.6	−20	11600	3.15×10^5	9.12×10^{10}
	13.5	−26	11900	3.24	9.67
	13.5	−33	12000	3.26	9.75
A. Cut perp. to vertical	20.85	−20	7600	3.17	9.2
	20.7	−26	7840	3.27	9.85
	20.7	−33	7900	3.29	10.0
B. Also perp. to the vertical —but in the 3rd rectilinear direction	20.6	−20	7620	3.14	9.06
	20.5	−26	7770	3.19	9.37
	20.5	−33	7800	3.20	9.40
Cut at 45° to the vertical	21.8	−20	7040	3.07	8.65
	21.7	−26	7150	3.11	8.92
	21.7	−33	7150	3.11	8.92

greater than the experimental error. The variation from sample to sample is probably not due to experimental error, but to differences in the elastic nature of the ice introduced sometime during its past history. Probably some differences in E in different directions will exist even if the rods are prepared under uniform conditions and preserved at a uniform temperature during the course of the experiment.

Discussion

A general statement by Swift (24) on the nature of elasticity, together with the work of McConnel and Kidd (15, 16) on the elasticity of ice, may adequately account for the variations shown in values of the elastic constant as given here.

According to Swift, "the deformation, which follows a change of static loading may, in the most general cases, be resolved into three separate constituents. (*a*) The strain which develops simultaneously with the change in stress, and of which it may be regarded as the cause or effect. This strain is essentially reversible in nature. (*b*) A deformation essentially permanent and irreversible which develops more slowly and with the passage of time approaches (in an irregular but roughly logarithmic way) its final value. (*c*) A strain which develops slowly in a similar way to the permanent set, but which is reversible. This strain is made evident in the partial recovery ("elastic afterstrain") which follows the removal of load from so-called semi-elastic materials, or from materials which have been overstrained, and in the reversible creep which occurs with elastic materials at high temperatures even under stresses which have apparently no permanent residual effect.

The processes which give rise to these changes are not known, but from the simple fact that strain may have this composite nature it follows that unless some regard is paid to the factor of time in the definition, the modulus of elasticity has no real physical significance and a restricted practical value."

The work of McConnel and Kidd indicates most clearly that in the case of

ice, the components (*b*) and (*c*) are ordinarily large parts of the whole strain, and may both be greater than (a); and that not only are (*b*) and (*c*) dependent on time, but also on temperature. Investigators determining the elastic modulus of ice probably were unaware of these facts and since no precautions seem to have been taken to avoid or make known these last two components agreement is not to be expected.

Again the work of McConnel and Kidd indicates a possible reason for the discrepancy between Matsuyama's results and those obtained in this investigation. They found that component (*b*) mentioned by Swift, varied with the manner of cutting the sample of ice with respect to the optic axis. Hence, unless precautions are taken to separate component (*b*) in each case the different determinations of E are not comparable.

The work of Swift with flexural vibrations in metal rods, and also the work of Pichot (18) on bars of rock, indicate that a dynamic method of determining E effectively eliminated factors (*b*) and (*c*). As has already been suggested, there is reason to believe that these general conclusions will be the same for longitudinal vibrations, for the stresses and strains take place in the direction of the long axis of the rod in both cases.

In brief, the work of McConnel and Kidd indicates that the weakness in a static method of elasticity determination applied to ice is that the components of deformation which are functions of time cannot be eliminated or made known. The work of Swift and Pichot, on the other hand, shows that by dynamic methods the components of deformation involving a time element may be eliminated and at the same time fairly accurate determination of the physical value of Young's modulus may readily be made.

As already mentioned, it was found in the present work on ice that there could be variations in E, at the same temperature, from sample to sample varying from 10.5×10^{10} to 9.0×10^{10} dynes/cm^2., even with rods cut from the same block of ice and in the same direction. But the differences in the values of E observed here are small in comparison with those obtained by static methods, though they are large in comparison with the experimental error of the present method and therefore cannot be so ascribed.

In this ultrasonic method the possible sources of error are:— (1) Error in measurement of the length of the ice rods; and (2) error due to possibly mistaking the identity of one mode or type of vibration for a totally different mode or type, *e.g.*, assuming that the fifth mode of transverse vibration is the first mode of longitudinal vibration; and (3) error in measurement of frequency. The errors in (1) and (3) together were not over 1%. An error due to a mistake in identity of one mode of vibration for another would result in value of velocity which was in error by some simple multiple of the true velocity. The transverse types of vibration were easily distinguished from the longitudinal types by the use of the stethoscope, for placing a stethoscope tip at the side of a bar when it was vibrating in a longitudinal fashion resulted in a sound very faint in comparison with that obtained by placing the tip at the end of the same vibrating rod. For the case of a transverse vibration the contrary was the case.

Hence the variation in velocity from sample to sample of ice of such magnitude as was shown here must be due to a real variation in the elastic properties of the samples. The discordant results quoted by Wyckoff (26) from the X-ray analysis of ice indicate that the crystal structure of ice may well be a function of its past history. A change in crystal structure in general must lead to a change in the elastic properties of the crystal, and though the continuous deformation of ice under constant stress observed by McConnel and Kidd also indicates that the crystal structure of river ice might be expected to be irregular, the results of this research indicate that the large differences in values of elastic constants formerly quoted cannot be considered correct.

References

1. BARTON, E. H. Text book on sound, 2nd ed. Macmillan. 1908.
2. BOYLE, R. W. and LEHMANN, J. F. Trans. Roy. Soc. Can. 111, 16: 115-125. 1927.
3. BOYLE, R. W. and FROMAN, D. K. Can. J. Research, 1: 405-424. 1929.
4. BOYLE, R. W. and REID, C. D. Trans. Roy. Soc. Can. 111, 20: 233-243. 1926.
5. BOYLE, R. W. and SPROULE, D. W. Nature, 123: 13. 1929.
6. BOYLE, R. W., TAYLOR, G. B. and FROMAN, D. K. Trans. Roy. Soc. Can. 111, 23: 187-201. 1929.
7. CHREE, C. Quart. J. Math. 21. 1886.
8. CLARKE, J. R. Phil. Mag. 4: 502-504. 1920.
9. HUBBARD, J. C. and LOOMIS, A. L. Nature, 120: 189. 1927.
10. KLEIN, E. and HERSHBERGER, W. D. Phys. Rev. 37: 760-774. 1931.
11. LAMB, H. Dynamical theory of sound, 2nd ed. Longmans. 1925.
12. LANG, R. J. Trans. Roy. Soc. Can. 111, 16: 163-173. 1922.
13. LOVE, A. G. H. Mathematical theory of electricity, 4th ed. Camb. Univ. Press. 1927.
14. MATSUYAMA, M. J. Geol. 28: 607-631. 1920.
15. MCCONNEL, J. C. Proc. Roy. Soc. 49: 323-343. 1891.
16. MCCONNEL, J. C. and KIDD, D. A. Proc. Roy. Soc. 44: 331-367. 1888.
17. MUZZEY, D. S. Phys. Rev. 36: 935-947. 1930.
18. PICHOT, M. J. Phys. radium, 6 ser. 8: 422-432. 1927.
19. PIERCE, G. W. Proc. Am. Acad. Sci. 60: 271-302. 1925.
20. PIERCE, G. W. Proc. Am. Acad. Sci. 63: 1-47. 1928.
21. POCHHAMMER, L. J. für Math., Crelle, 881: 324. 1876.
22. QUIMBY, S. L. Phys. Rev. 25: 558-573. 1925.
23. RAYLEIGH, LORD. Theory of sound, Vol. 1. Macmillan. 1894.
24. SWIFT, H. W. Phil. Mag. 2: 351-368. 1926.
25. THORNTON, W. M. Phil. Mag. 38: 705-707. 1919.
26. WYCKOFF, R. W. G. Structure of crystals, Chem. Cat. Co. 1924.

VELOCITY OF SOUND IN CYLINDRICAL RODS[1]

By Geo. S. Field[2]

Abstract

The experimental knowledge so far available of the velocity of longitudinal waves in cylindrical rods is reviewed, and it is shown that a close analogy most probably exists between waves in cylinders of liquid and in solid rods. The theory for rods due to Pochhammer is considered with reference to a specific case for which experimental velocities have been determined, and it is shown that the agreement at low frequencies is good. At higher frequencies, however, theory and experiment differ widely.

Introduction

In view of the results recently obtained (5), both experimentally and theoretically, in connection with the propagation of sound through liquids contained in cylindrical tubes, it was decided to investigate the possibility of similar results being obtained with solid cylindrical rods. It is to be expected that the introduction of an extra constant (Poisson's ratio) will modify the results, but we may look for the phenomenon of selective absorption at and near the frequencies of radial resonance for rods made of isotropic materials; and even with anisotropic materials something of the kind will most probably occur.

As a matter of fact, experiments which have already been performed have indicated that in the frequency ranges studied the velocity-frequency relation for solid rods is analogous to that for cylinders of liquid. In a paper by Boyle and Sproule (4) it is shown that at low frequencies, where $\left(\frac{rk}{l}\right)^2$ is a small fraction (r is the radius of the rod, l the length and k the mode of vibration), the velocity is not very different from $c_0 = \sqrt{\frac{E}{\rho}}$, where E is Young's modulus of elasticity and ρ is the density. The correction suggested by Rayleigh,(7, pp. 251-3) applies at slightly higher frequencies, but, for example, in duralumin when $\left(\frac{rk}{l}\right)^2 > 0.3$ the velocity decreases so rapidly with frequency that this correction is no longer applicable. The rapid drop in the velocity-frequency curve near a certain frequency, characteristic of the material and dimensions of the rod, is the same phenomenon that was observed for liquids in the region of anomalous dispersion. There then follows a range of frequencies for which no experiments have been conducted, but at very high frequencies, where $\left(\frac{rk}{l}\right)^2$ is very great, the researches of R. W. Boyle and coworkers (2, 3) have shown that the velocity is very high, approximately what would be given by taking the velocity equal to $\sqrt{\frac{K}{\rho}}$, where K is what Barton (1, pp. 125, 190) calls the "elongational

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elasticity", and is to be used for elongational waves in an infinite medium. This value of velocity corresponds to the value found for cylinders of liquid when the wave-length was very small compared with the radius, *i.e.*, when the liquid behaved as if it were an infinite medium.

Experiments are now under way in this laboratory to investigate the frequencies above the range where the velocity is very low, and it is hoped soon to demonstrate the complete phenomenon from very low frequencies to very high, corresponding to values of $\left(\frac{rk}{l}\right)^2$ from zero to infinity. In the meantime, existing theory has been closely examined to see if it will explain what is already known and perhaps indicate what is to be expected in the range where experiments are now being conducted.

A short time ago Ruedy (8) published an analysis of the theory due to Pochhammer (6) and showed that the velocity of a longitudinal wave in a solid rod would decrease steadily with increasing frequency. It was suggested that anomalous dispersion of sound waves would probably occur at the resonant frequencies of the radial vibrations, but no mention was made of the effect this phenomenon would have on the velocity.

In the following paper Pochhammer's equations are put into a form similar to those recently obtained for the velocity of sound in liquids contained in tubes. The equations are then applied to the case of a duralumin rod, for which some experimental data are available, and conclusions are drawn as to the probable accuracy of the theory.

Velocity Equation

The theory gives the following expression for the propagation of waves along a rod,

$$\left\{2\gamma^2-\frac{p^2\rho}{\mu}\right\}J_1(k'a)\left\{J_1(h'a)\,\frac{2\mu h'}{a}-J_0(h'a)\left[\frac{p^2\rho\lambda}{\lambda+2\mu}+2\mu h'^2\right]\right\}$$
$$=-4\gamma^2h'\mu J_1(h'a)\left\{-\left(\frac{1}{a}\right)J_1(k'a)+k'J_0(k'a)\right\}, \qquad (1)$$

where $\gamma^2=\frac{p^2}{c^2}$, $p=2\pi$ times the frequency (n), $c=$ phase velocity of the wave, $\rho=$ density of the material, $a=$ radius of the rod, $\mu=\frac{E}{2(1+\sigma)}$, $\lambda=\frac{E\sigma}{(1+\sigma)(1-2\sigma)}$, $E=$ Young's modulus, $\sigma=$ Poisson's ratio, $h'^2=\frac{p^2\rho}{\lambda+2\mu}-\gamma^2$, $k'^2=\frac{p^2\rho}{\mu}-\gamma^2$, and J_0 and J_1 are Bessel's functions of the first kind of order zero and one.

If we substitute $\gamma^2=\frac{p^2\rho}{\lambda+2\mu}-h'^2$ and write $h'a=x$ and $k'a=y$, Equation 1 may be rearranged to give,

$$x\frac{J_1(x)}{J_0(x)}=\frac{\left\{2x^2+\frac{a^2p^2\rho\lambda}{\mu\lambda+2\mu^2}\right\}^2}{\frac{2a^2p^2\rho}{\mu}+4\left\{x^2-\frac{a^2p^2\rho}{\lambda+2\mu}\right\}y\frac{J_0(y)}{J_1(y)}}. \qquad (2)$$

Since $h'^2-k'^2=p^2\rho\left\{\frac{1}{\lambda+2\mu}-\frac{1}{\mu}\right\}$, we have an equation relating x and y

as follows,

$$y^2 = x^2 + \frac{a^2 p^2 \rho(\lambda+\mu)}{\mu\lambda+2\mu^2} . \tag{3}$$

Now $\frac{p^2}{c^2} = \gamma^2 = \frac{p^2\rho}{\lambda+2\mu} - h'^2 = \frac{p^2\rho}{\lambda+2\mu} - \frac{x^2}{a^2}$. (4)

And, $\frac{\lambda+2\mu}{\rho} = \frac{E}{\rho}\left\{\frac{\sigma}{(1+\sigma)(1-2\sigma)} + \frac{1}{1+\sigma}\right\} = \frac{E}{\rho}\left\{\frac{1-\sigma}{(1+\sigma)(1-2\sigma)}\right\}$. (5)

Hence, if we put $c_0^2 = \frac{E}{\rho}$, we have,

$$\frac{p^2}{c^2} = \frac{p^2}{c_0^2}\left\{\frac{(1+\sigma)(1-2\sigma)}{1-\sigma}\right\} - \frac{x^2}{a^2} . \tag{6}$$

Rearranging, and writing $2\pi n$ for p, we obtain,

$$c^2 = \frac{c_0^2 n^2}{\frac{(1+\sigma)(1-2\sigma)}{1-\sigma} n^2 - \frac{x^2 c_0^2}{4\pi^2 a^2}} . \tag{7}$$

From Equation 2 it is possible to obtain x, and from Equation 7 the phase velocity, c, may then be determined.

It will be seen from the definition of h' and k' that these two quantities may be real or imaginary, depending upon the value of γ, which in turn is dependent upon phase velocity.

Equations Applied to a Specific Case

Consider a duralumin rod, with constants as follows: $a = 2.55$, $c_0 = 5.2 \times 10^5$, $\sigma = 0.36$, $\rho = 2.7$, $E = 7.3 \times 10^{11}$.

$$\text{Now,} \quad h'^2 = p^2\left\{\frac{\rho}{\lambda+2\mu} - \frac{1}{c^2}\right\} . \tag{8}$$

$$\text{And} \quad k'^2 = p^2\left\{\frac{\rho}{\mu} - \frac{1}{c^2}\right\} . \tag{9}$$

Substituting the numerical values, we have,

$$h'^2 = p^2\left(\frac{1}{(1.3c_0)^2} - \frac{1}{c^2}\right) \tag{10}$$

$$k'^2 = p^2\left(\frac{1}{(0.61c_0)^2} - \frac{1}{c^2}\right) . \tag{11}$$

From these equations we see that, since $h' = \frac{x}{a}$ and $k' = \frac{y}{a}$, we have three possibilities:

(a) if $c < 0.61\ c_0$, then x imaginary and y imaginary,

(b) $1.3c_0 > c > 0.61c_0$, then x imaginary and y real,

(c) $c > 1.3\ c_0$, then x real and y real.

For low frequencies we know experimentally that c approximately equals c_0. In any case it is in the range given by (b). Hence to begin with we use x imaginary and y real.

If the numerical values are then substituted in Equation 2, this equation becomes,

$$x' \frac{I_1(x')}{I_0(x')} = \frac{\{x'^2 - 7.3n^2\}^2}{-13n^2 + (x'^2 + 5.7n^2)y \dfrac{J_0(y)}{J_1(y)}}, \tag{12}$$

where $ix' = x$, $J_0(ix') = I_0(x')$, $J_1(ix') = iI_1(x')$, and the frequency, n, is expressed in units of 10^5 cycles per sec.

From Equation 12 values of x' were determined for different frequencies as given in Table I.

TABLE I

SOLUTIONS OF EQUATION 12 FOR $1.3c_0 > c > 0.61c$

n in units of 10^5	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8
x'	0.20	0.40	0.66	0.95	1.33	2.0	2.76	3.46

At frequencies of 90000 cycles/sec. and higher, for the rod under consideration, it is found that for a solution of x it is necessary to use Equation 12 with both x and y imaginary, corresponding to $c < 0.61\ c_0$. In the equation this means using $y' \dfrac{I_0(y')}{I_1(y')}$ instead of $y \dfrac{J_0(y)}{J_1(y)}$, where $y = iy'$; otherwise Equation 12 is unaltered.

Values of x' for the higher frequencies were then determined as in Table II.

TABLE II

SOLUTIONS OF EQUATION 12 FOR $c < 0.61c_0$

n in units of 10^5	0.9	1.0	1.2	1.4	2.0
x'	4.08	4.70	5.77	6.82	9.84

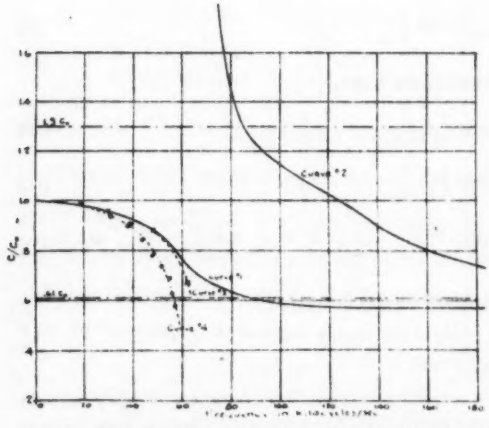

FIG. 1. *Velocity of sound in a cylindrical duralumin rod, showing theoretical and experimental curves.*

By substituting for x in Equation 7, and remembering that $(-x^2) = -(ix')^2 = +x'^2$, the velocity corresponding to each x' was determined. A curve of velocity against frequency was then plotted as in Fig. 1 (Curve No. 1).

If anomalous dispersion were occurring, we should expect a higher velocity than c_0 to exist for frequencies above the absorption frequency. Accordingly, solutions for Equation 2 were sought in the range $c > 1.3c_0$; that is, with x and y real. Table III shows the results.

TABLE III

SOLUTIONS OF EQUATION 12 FOR $c > 1.3\ c_0$

n in units of 10^5	0.75	0.80	0.83
x	1.23	.78	.45

For frequencies of 85000 and over, in order to obtain a solution it was necessary to use Equation 2 for the range $1.3c_0 > c > 0.61\ c_0$; that is, x imaginary and y real. Values of x' so determined are given in Table IV.

TABLE IV

SOLUTIONS OF EQUATION 12 FOR $1.3c_0 > c > 0.61c_0$

n in units of 10^5	0.9	1.0	1.1	1.2	1.3	1.4	1.5	1.8	3.0	6.0
x'	0.74	1.27	1.72	2.2	2.79	3.46	4.18	6.22	12.5	26.7

By using Equation 7 and the values of x' and x from Tables III and IV, velocities were determined for the corresponding frequencies, and were plotted in Fig. 1 (Curve No. 2).

TABLE V

Resonant frequency in kilocycles/sec.	Supposed harmonic	Velocity of wave in cm./sec.
22.7	5	4.88×10^5
30.8	7	4.73
37.6	9	4.50
38.3	9 (?)	4.58
43.3	11	4.23
47.2	13	3.91
48.2	13 (?)	3.98
51.6	15	3.70
54.8	17	3.46
55.6	19	3.17
56.9	21	2.89
57.5*	—	—
58.1*	—	—
59.1	?	?
59.7	?	?
61.0	?	?
62.0	?	?
65.3	?	?
Etc.	?	?

**Continuous resonance—probably here velocity so low that harmonic frequencies are too close to be separable.*

On the same figure (Curve No. 3) have been plotted for comparison a few velocities obtained *experimentally* by Boyle and Sproule (4) for the duralumin rod considered above. Another experimental curve (No. 4) is shown which was obtained for a cylinder of somewhat larger radius (3.38 cm.), as this curve shows somewhat better the very rapid decrease in velocity which occurs near a definite frequency for each cylinder. The experimental velocities determined by method (5) of Boyle and Sproule (4) for a number of frequencies, are given for this case in Table V, and it is there shown that a discontinuity exists at a frequency in this case of about 57 kilocycles/sec.

Conclusions

From what is experimentally known about the velocity of sound in rods, any theory which purports to explain the facts should give certain velocity-frequency relations as follows:

(1) Starting with a value c_0 at very low frequencies, *i.e.*, $\left(\frac{rk}{l}\right)^2$ a small

fraction, the velocity should decrease steadily with frequency, until,

(2) At a certain frequency the velocity is decreasing so rapidly that it is impossible to obtain resonance points with a rod, and an apparent discontinuity in the velocity-frequency curve exists.

(3) At very high frequencies the velocity in the rod is comparatively high, being of the order of what would be given by using the elongational modulus of elasticity instead of Young's modulus in the equation $c = \frac{E}{\rho}$.

The theory due to Pochhammer, as has been demonstrated in the preceding analysis, indicates a decreasing velocity with increasing frequency, and the agreement at the lower frequencies with experimental determinations is very good. However, there is no discontinuity in the theoretical curve, and the velocities at very high frequencies are not greater than c_0, but much less. It is to be noted, therefore, that the theory which has been discussed accounts for only one, *i.e.*, the first, of the three velocity-frequency relations which are already experimentally known.

Added Note

That the high velocities at high frequencies mentioned in experimental velocity-frequency relation No. 3 are not obtainable from Pochhammer's equations may be shown in the following way:

If $x = 0$ in Equation 7, the velocity is the same as is given by Barton (1, pp. 125,190) for an infinite medium, *i.e.*, equivalent to $c = \sqrt{\frac{\text{elongational modulus}}{\text{density}}}$. This is the velocity that experimentally has been found to occur at high frequencies, where $\left(\frac{rk}{l}\right)^2$ is very large.

Substituting $x = 0$ in Equation 12, we get,

$$0 = \frac{53.5n^4}{-13n^2 + 5.7n^2 \left(4.55n \frac{J_0(4.55n)}{J_1(4.55n)}\right)}.$$

But this equation, although satisfied at intervals, *i.e.*, when $\frac{J_0(4.55n)}{J_1(4.55n)} = \infty$, is not satisfied for a *range* of high frequencies (in fact, if correct it should be satisfied for *all very high* frequencies, as the rod should then behave as an infinite medium, and has been shown so to do (2, 3).

In conclusion, therefore, the theory accounts for the right velocity when $n \longrightarrow 0$, but does not do so when $n \longrightarrow \infty$.

References

1. BARTON, E. H. A text book on sound. Macmillan, 1908.
2. BOYLE, R. W. and FROMAN, D. K. Can. J. Research, 1: 405-424. 1929.
3. BOYLE, R. W. and SPROULE, D. O. Can. J. Research, 2: 3-12. 1930.
4. BOYLE, R. W. and SPROULE, D. O. Can. J. Research, 5: 601-618. 1931.
5. FIELD, G. S. Can. J. Research, 5: 132-148. 1931; Nature, 128: 117. 1931.
6. POCHHAMMER, L. J. Math. (Crelle), 81: 324-336. 1876. (Summarized by A. E. H. Love in a treatise on the mathematical theory of electricity. Camb. Univ. pr. 1927.
7. RAYLEIGH, LORD. Theory of sound, 2d ed. v. 1. Macmillan. 1894.
8. RUEDY, R. Can. J. Research, 5: 149-155. 1931.

THE IONIZATION OF THE ATMOSPHERE MEASURED FROM FLYING AIRCRAFT[1]

By D. C. Rose[2]

Abstract

The Gerdian type of atmospheric ionization measuring apparatus was attached to a cabin aeroplane so that the state of ionization of the atmosphere could be studied. The limitations of the apparatus for aeroplane use are discussed. Measurements were taken from ground level to heights of 15000 ft. The results are plotted in number of ions per cc. (separate curves for positive and negative) at different altitudes.

The results indicate that at the cloud level there is an abnormal excess of small positive ions and a minimum in the excess of positive ions over negative ions from 4000-6000 ft. higher. This does not include large ions such as charged water drops or dust particles. The observations were taken in regions free from clouds, the cloud level being determined by observation on clouds in the sky, and by relative humidity measurements taken at the same time.

A great deal of work has been done on the conductivity or state of ionization of the atmosphere at ground level*. Diurnal and yearly variations have been found both on land and at sea for which no adequate explanation has been brought forward. The present paper deals with an attempt to study the variation in the ion content of the air at different altitudes. Little attention has been paid to the absolute value of the number of ions per cc. in the air but the relative values at different altitudes have been studied with a view to correlating variations in the ionic content of the atmosphere with weather conditions. This work was undertaken in connection with some work on the elimination of static charging of the films in the cameras used for aerial photography, for which it was found advisable to measure relative humidity and other atmospheric conditions in the slip stream of flying aircraft. The results of the relative humidity experiments are published elsewhere (5).

Method of Measurement

The instrument used for measuring the state of ionization in the air was the Gerdien conductivity apparatus. A sketch of the apparatus as used for these experiments is shown in Fig. 1. The ion tube was of brass, 5.43 cm. in diameter and about 14 in. long. The collecting electrode was a copper rod the effective length of which was taken as 29.2 cm., the diameter being 0.63 cm. The collecting electrode was connected to a Wulf type of fibre

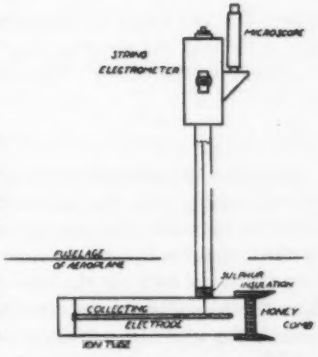

FIG. 1. *Apparatus used for measuring the conductivity of the air.*

*Publications of the Department of Terrestrial Magnetism, Carnegie Inst., Washington.

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electrometer by a fine wire tightly stretched through the centre of a side tube brazed to the ion tube as shown in the figure. The function of the honeycomb and the position of the apparatus in the aeroplane will be described in a latter section.

Theory and Method of Reduction of Observations

The theory of this type of ion measuring apparatus has been considered carefully by Swan (8). He shows that for the general case the rate of loss of an insulated charged body in a stream of air is given by the equation:

$$-\frac{dQ}{dt} = 4\pi QneM, \tag{1}$$

where Q is the charge on the central collector, n the number of ions per cc. in the air (assumed sufficiently small that it has no effect on the electric field), e the charge on the ions and M the mobility of the ions. To put the equation in a working form Swan has shown very simply that when the insulated rod and part exposed to the air has a measured capacity C, and the total insulated system (rod, electrometer element and connections) C_1, the equation may be reduced to the form:

$$-C_1\frac{dV}{dt} = 4\pi CVneM \tag{2}$$

where V is the potential of the insulated system.

Actually the capacity of the insulated system was not measured, but treating it as a cylindrical condenser and neglecting corrections for the end of the rod and for the parts connecting it to the electrometer which are exposed to the air, the equation may be written:

$$-C_1\frac{dV}{dt} = \frac{2\pi neMLV}{\log\frac{b}{a}}, \tag{3}$$

where L is the length of the collecting rod and b and a the radii of the ion tube and collecting rod respectively. This equation can be integrated easily but in the present experiment it was used in the differential form:

$$-ne = \frac{C_1\log\frac{b}{a}}{2\pi MLV}\frac{dV}{dt}. \tag{4}$$

As Swan has pointed out, this formula as used originally by Gerdien is only approximately true and unless the length of the tube and electrode is many times its diameter the error in using the above formula may be large. In the present case the error would be much smaller than in the case described by Swan (error of 39%) as the tube was much longer in comparison to its diameter. Also these results are necessarily of a preliminary nature as only four flights were taken and the adaption of these ionization measuring instruments to aeroplane use was by no means simple. Other sources of error will be discussed below.

If we call M_0 the mobility of the ions concerned at a temperature t_0 and pressure p_0 the value of M in Equation 4 is:

$$M = M_0\frac{P_0}{P}\frac{t}{t_0}$$

Further, putting S equal to the sensitivity of the electrometer in electrostatic units per division Equation 4 becomes:

$$-ne = \frac{C_1 \log \frac{b}{a} Pt_o S}{2\pi M_o P_o t L V} \frac{dx}{dt}, \tag{5}$$

where x is the scale reading of fibre in the electrometer. The value of C_1 was found by measurement to be 37.4×10^{-12} farads. It was measured by the method of mixtures using a calibrated Leybolds cylindrical condenser as a standard and the electrometer itself as a potential measuring device. P was measured throughout the flight by a Tycos aneroid barometer. The temperature was taken from the dry bulb thermometer used in the humidity measuring device carried alongside the ion tube.

The procedure followed in taking readings and working out the results was as follows: During the flights, with the exception of the first, the plane was flown level at the altitude being studied for a period long enough to take four or five good readings of the position of the electrometer fibres on the microscope scale. Then the plane was flown to the next level, the insulated system recharged and another set of readings taken. In plotting the results the actual readings of the electrometer were plotted for each altitude. A tangent was drawn to a smooth curve through the points (usually nearly a straight line) and the slope of the tangent was measured. This gives a value of $\frac{dx}{dt}$ at the same value of x for each altitude or at the same value of the potential. Reducing the results in this manner the potential at which the observation is taken may be considered as constant during the particular flight in question. and the relative value of the quantity ne can be calculated from the simpler relation:

$$ne = K \frac{Pt_o}{t} \frac{dx}{dt}, \tag{6}$$

K being a constant which changes for each flight and for different varieties of ions. The quantity plotted in Fig. 2 is the value of $\frac{Pt_o}{t} \frac{dx}{dt} \times 10^{-3}$, P being in millibars and $\frac{dx}{dt}$ in divisions per minute: t_o was not included in the value of K for the sake of simplicity in calculation because when the temperature t did not vary by more than about 10° C. the temperature correction was neglected as it would be smaller than other possible errors.

Mobility

To obtain a measure of the number of ions per cc. in the air it now remains to evaluate K. The only quantity which cannot be determined easily is the value of M_o, the mobility at N.T.P. A review of recent literature on mobilities of ions in air shows that there are still conflicting results. The mobility as measured in the laboratory varies not only with pressure and temperature but with humidity, purification of the air, potential, etc. Also, without doubt there is more than one type of ion of the same sign involved.

The difficulty might be overcome by plotting the results in positive and

negative conductivity, as is more often done, rather than number of positive or negative ions per cc. In the present case, as the variation in space charge with altitude was being studied, it seemed more appropriate to assume the most likely mean mobility and evaluate K from it. From the work of Zeleny (13), Hamshere (1) and Nolan and Nevin (4), it seems reasonable to assume a mean mobility of 1.8 for negative ions and 1.2 for positive ions and the results are computed on that basis. It should also be pointed out that heavy ions found in the atmosphere, such as the dust particles, are also omitted in this discussion as the apparatus would collect only a negligible number of them.

Aerodynamics of the Ion Tube

Before proceeding with the results there are some limitations to the application of this Gerdien type of conductivity or space charge apparatus. First assuming a streamline flow in the tube there is a definite radius r_0 of a cylinder of the air passing through the tube from which all the ions are collected. The value of r_0 must be less than b, the radius of the ion tube, otherwise the tube becomes saturated and the number of ions per cc. then depends on the velocity of the air through the tube bearing a relation totally different from Equation 5. As the velocity of air in the tube was not measured it is not advisable to allow this to take place. It may be shown that the value of r_0 is given by the equation:

$$r_0^2 = \frac{2M\ VL}{v \log \frac{b}{a}}, \tag{7}$$

where v is the velocity of the air stream. Swan uses $(r_0^2 - a^2)$ in place of r_0^2 on the left side of the equation and assumes that all the ions in the cylinder of radius a strike the end of the collecting rod. If the air flow around the rod is streamline, they probably do not because the air within the cylinder of radius a would be spread and only a few of the ions in it would strike the front end of the rod. A more correct value for the left hand side of Equation 7 is $(F^2 r_0^2 - a^2)$, where F is a function slightly greater than unity. This correction, however, can be omitted as when r_0 is of the order of b, a^2 can be neglected. A rough calculation of the limit of r_0 is all that is required.

The aeroplane was normally flown at a speed of between 70 and 80 miles per hour. The ion tube was in the slip stream which would have a considerably higher velocity than that of the plane, but the speed of the air through the tube would not be as high as that of the stream in which it was placed. Hence, taking the speed to be about 70 miles per hour, under average conditions, the value of r_0 from Equation 7 was about 2 cm. which was quite satisfactory. At the highest altitudes, where the mobility of negative ions is increased due to the low pressure, the value of r_0 becomes dangerously near the upper limit ($b = 2.7$ cm.) for which Equation 5 is applicable. Any error in this calculation due to the assumption of too low a mobility would mean that the results for the number of negative ions per cc. at high altitudes are too low.

Swan has shown that it is not necessary for the air in the ion tube to have a constant velocity over its cross section. However, when the air in the tube

becomes very turbulent, Equation 5 can no longer hold. The velocity in the tube would vary enormously with time, cross section, and length and the conditions that $r_o < b$ would be meaningless. The condition for streamline flow in such a tube is that Reynolds' Number, R, should be less than about 1500. Reynolds' number is given by

$$R = \frac{vd}{u},$$

where v = velocity in feet per sec., d = diameter of opening in feet and u = the kinematic coefficient of viscosity, usually taken as 0.000159. At 70 miles per hour R becomes about 121,000 for this tube*. Hence the air flow in the tube was very turbulent.

In order to remove this turbulence and make the airflow in the tube such that the state of ionization could be measured by the relations discussed above, a honeycombed opening was designed to fit the intake end of the ion tube as shown in Fig. 1. It consisted of $\frac{1}{8}$-in. square openings placed in a streamline cone the inside diameter of which was the same as the inside diameter of the ion tube. No doubt the honeycomb had some effect on the value of the results. Scholz (6) has done some experiments using this type of ion tube. Although his experimental conditions were somewhat different from those described here, he showed that the passage of the air through other tubes before it reaches the collecting electrode has some effects on the results. However, the error would be in the absolute value, not in the relative values at different altitudes. Probably the honeycomb does not remove all turbulence as there may be some in the smaller openings but the resulting flow through the tube would be laminar and hence Equation 5 should apply.

The Electrometer

The electrometer was of the fibre type. For Flights 1, 2 and 3 double fibres were used, the separation of the fibres being measured on a scale in the eyepiece of a microscope. The tension on the fibres was maintained by a quartz bow. The fibres were connected to the insulated system, the insulation throughout being of sulphur. The knife edges together with the ion tube and shields were earthed to the frame of the aeroplane. Sensitivities of from 6 to 10 volts per division at potentials of about 200 volts were easily obtainable and were found very satisfactory. Much greater sensitivities could be obtained at lower voltages by applying potentials to the knife edges but this would involve the carrying of a large number of batteries. The insulated system was charged by a 90-volt battery which charged a variable condenser. The battery was then disconnected, the capacity of the condenser was reduced until the insulated system reached the required potential (potentials of 400 or 500 volts could be obtained easily in cold weather by this means but the instrument was usually operated between 150 and 250 volts), then the condenser was disconnected from the insulated system.

On the fourth flight, a different type of fibre suspension was used having

**Dr. J. J. Green of the Aeronautical Section of the National Research Council kindly helped the author in this phase of the problem.*

only one fibre which, with one knife edge, acted as the insulated element of the electrometer. This was found more satisfactory to construct and calibrate but gave more trouble due to vibration. Although at all times the vibration of the aeroplane made the readings very difficult, usually with the double fibre suspension good readings could be obtained without disturbing the normal course of flight. In the case of the single fibre suspension the vibration was much more noticeable and in some cases readings could be taken only by arranging for the pilot to throttle down the engine and glide for the few seconds required. The ion tube, with the other apparatus, was suspended through the camera hole in the floor of the aeroplane. It was supported by rubber tubing with the intention of reducing the vibration to a minimum. The supports, however, were not entirely satisfactory as the above discussion indicates.

The electrometer was calibrated in the laboratory before and after each flight, but the accuracy of calibration was never very great because usually it was found to have changed slightly each time due to the rough treatment necessary in installing the instrument in the plane. The same fibre was used in the first two flights but it did not survive the dismantling after the second. In Flights 3 and 4 the fibre was again broken before it could be recalibrated after the flight. This places some doubt on the absolute value of the number of ions per cc. but by comparing different calibrations it is estimated that the error should not be greater than 10 or 15%. The relative values of course are much more accurate as the value of $\frac{dx}{dt}$ was determined at the same deflection for each point on the curves, and there is every reason to believe that the calibration remained reasonably constant during flight.

Charge on Aircraft

Another possible influence on the results of these observations should be mentioned. Wigand (10) has shown that aeroplanes will acquire a positive charge while in flight due to the fact that the exhaust gases from the engine are negative. This would attract negative ions to the aeroplane and give a result which might show the right variations with altitude but wrong absolute values. However, it is unlikely that this charge has any effect because of the velocity with which the plane moves through the air. The ion tube was about three metres from the front of the plane and about 15 cm. from the fuselage. At a velocity of 25 metres per sec. through the air, the potentials around the fuselage would have to be very high indeed to create sufficient potential to move the ions 15 cm. during the time (about $\frac{1}{8}$ sec.) the air stream flows from the front of the plane to the position of the ion tube.

The action of the propeller on the air may also have had some effect on the results. McDiarmid (3) measured the charge on an insulated tube when dry dust-free air flows through it rapidly. Her results showed a charging of the tube and a corresponding charge of opposite sign in the air. The direction of the sign depended on the material of which the tube was made. In the case of aluminium the charge was usually negative, leaving the air positive. Hence in these experiments the propeller (aluminium alloy) moving through the air

might have some effect on the results. The magnitude of this effect would be difficult to estimate though it would not be expected to be great. It should have been eliminated by placing the ion tube out of the slip stream on a wing strut or some such part of the plane. It would be very difficult to do this because of the necessity of having the electrometer in the cabin. It was therefore not attempted during these flights.

Results

The results of the observations taken during flight have been reduced to the curves shown in Fig. 2 by the methods outlined in the previous sections. The abscissas represent the altitude in thousands of feet. This was read in two ways, on the aircraft altimeter and on the Tycos aneroid barometer carried beside the ion tube. A reduction of the barometer readings to feet agreed within 100 or 200 ft. with the altimeter readings in Flights 3 and 4 but showed considerable disagreement in Flights 1 and 2. The barometer readings, however, were assumed to be the more accurate. In the case of the ordinates, the figures on the left of the vertical axis represent the value of $\frac{Pt_0}{t}\frac{dx}{dt} \times 10^{-3}$ as indicated in Equation 6. It is plotted this way because of the fact that the absolute values of n cannot be known nearly as accurately as the relative values. The higher figures inside the axis represent the value of n in number of ions per cc., assuming that each ion has a charge equivalent to one electron and values for mobilities as discussed above. The curves are drawn by joining the points by straight lines.

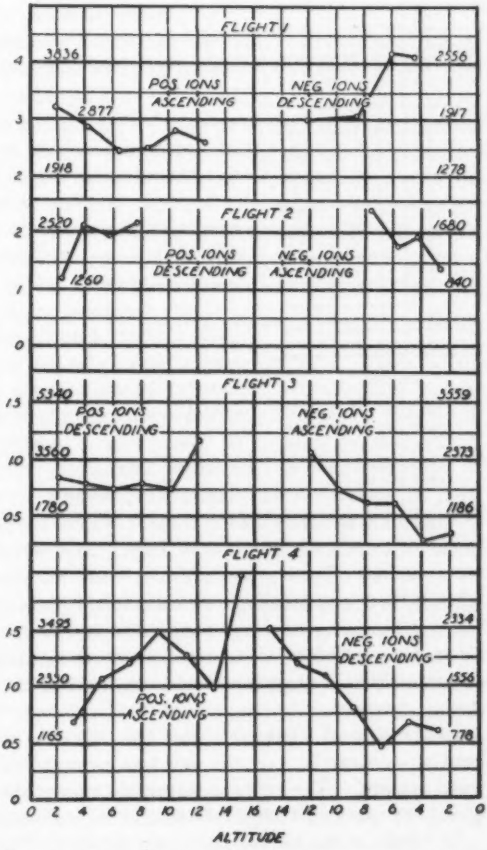


FIG. 2. *The state of ionization of the air at different altitudes. The ordinates are expressed in arbitrary units and in number of ions per cc.*

The curves representing the results of Flights 2, 3 and 4 show considerable similarity, particularly Flights 3 and 4, while Flight 1 is totally different. The reason for this, as mentioned in a previous section, is that in Flight 1 the ion tube was not equipped with the honeycomb. For this reason it will not be included in further discussion. As would be expected, the number of positive ions per cc. is greater than the number of negative. As one ascends, the number of ions of both signs shows a general tendency to rise, but the number of negatives rises more rapidly at first than the number of positives. This is more readily seen if one plots the difference between the number of positive and number of negative ions per cc. at different altitudes. This has been done in Fig. 3.

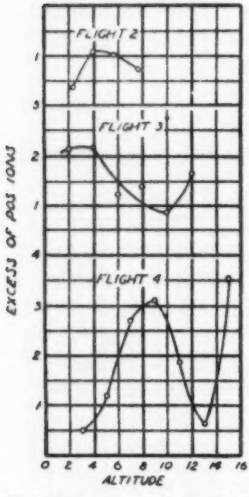


FIG. 3. *Excess of positive ions over negative. The ordinates represent thousands of ions per cc.*

Suppose we examine first the results of Flight 4. The excess of positive ions increased until an altitude of 9000 ft. was reached, then decreased rapidly to a minimum at 13000 ft. The shape of the curve for Flight 3 shows the same maximum and minimum as in the case of Flight 4 at altitudes of about 3000 and 9000 ft. respectively. In the case of Flight 2, an insufficient number of points was taken though the curve does indicate a maximum at about 4500 ft. A comparison of these results with weather conditions and the relative humidity (5) curves taken during the same flights is interesting.

Flight 2 was taken during a day (March 2, 1931) on which there was a thin layer of clouds about 3000 ft. high, from which occasional flurries of snow were falling. During the flight, clouds were avoided as much as possible. The sky was not totally overcast so clear places could always be found. The relative humidity readings show a maximum at about 4000 ft. (The aircraft altimeter read 5000 ft. but the barometer recorded a pressure which indicated about 4000 ft. Negative ions were studied during ascent and positive ions during descent.

In the case of Flight 3 (April 18, 1931) the record of the weather was not kept so accurately, but the sky was either clear or very few cumulus clouds were present. The relative humidity curves show a maximum at 4000 ft. or about the same altitude as the maximum excess of positive ions.

In Flight 4 the maximum in the curve in Fig. 3 again occurs at the same altitude as the relative humidity peak. The weather during this flight (June 17, 1931) was fine but there were numerous cumulus clouds at altitudes between 7000 and 9000 ft. During the flight these clouds were avoided as much as possible and in no case was the plane flown through or near them.

Although no great accuracy can be expected from these results, as has been indicated in the descriptions of the method of measurement, these three flights

were taken under sufficiently different conditions and the results are sufficiently consistent to show a definite indication that there is some change in the ionic content of the air about the cloud level. The change manifests itself by a decrease in the excess of the number of positive over the number of negative ions and the maximum of that excess appears just at the cloud level. A minimum in that excess then appears 4000 to 6000 ft. higher.

Conclusions

To compare the results of these experiments with others is difficult because as far as the author is aware no similar observations have been taken under similar circumstances. The great majority of ionization measurements have been made on the ground. Wigand in 1914 (11) and in 1921 (9) measured the conductivity of the air with a similar apparatus during flights in a manned balloon and found that in general the conductivity increased with altitude, the positive conductivity remaining higher than the negative. This does not necessarily mean that the number of ions per cc. increases with altitude as the conductivity follows the relation $\lambda = neM$ and the mobility M increases with reduced pressure. His results show that at levels where there is mist the value of $\frac{\lambda +}{\lambda -}$, the ratio of positive to negative conductivities, usually assumes a lower value than in clear places, often going below unity. These experiments are in agreement with the results of the present investigation in that the number of positives were apparently decreased relative to the number of negatives in or just above clouds.

Lugeon (2) measured the number of ions per cc. in the air at altitudes of 2450 and 4358 metres (8040 and 14300 ft.) and found that in daytime the total number of ions was less at the higher level than at the lower. His observations were taken on mountains and therefore would be expected to differ somewhat from those in free air due to the influence of the mountain. He records that as a cloud came up the mountain past the station, a greater excess of positive ions was noted above the cloud and relatively more negatives were found in the cloud. In the present experiments no visible cloud existed where measurements were taken but there is an indication of a cloud level in the humidity curves. It will appear later that it is difficult to compare his results with those obtained by the author because it is not clear whether or not Lugeon's results included both heavy and light ions.

Wigand (10) made some more recent measurements of potential gradient from which the resulting space charge can be deduced by using Poisson's equation. His measurements were taken during flights in an airship. He found that normally the potential gradient on the ground is positive and decreases in such a way that the space charge diminishes with height. On one of his ascents he found an inversion in the potential gradient slope which indicates an excess of negative space charge between 700 and 800 metres altitude (2296 and 2625 ft.). At higher altitudes the resulting space charge became small. This inversion in the space charge or a decrease in the excess of positive ions in or near cloud levels was noticed in early experiments by Elster and

Geitel, and Linke, which are discussed by Wigand. They introduced a theory for this inversion based on the normal upward movement of negative ions and downward movement of positive ions. These ions when coming into a region of mist or haze become attached to heavy particles and their normal mobility becomes greatly reduced. Hence at the top of a layer of mist or haze there would be an accumulation of positive ions and at the bottom an accumulation of negative ions. Wigand on one flight noted the accumulation of negative ions at the bottom, but found no corresponding excess of positive at the top. As ascents were not above 1300 metres, he may not have been high enough. It is obvious that this action could not go on for any length of time as the electric fields built up would tend to neutralize it.

In the present experiments the peak in the curves representing the excess of positive ions over negative may be more apparent than real because of the greatly lower mobility of heavy ions. The apparatus used would measure a negligible number of heavy ions, so if at the cloud level the negative ions were made ineffective by condensation, the results would indicate a greater excess of positives in a region where there might really be considerable negative space charge as well as positive. The minimum in the curves in Fig. 3 might be explained in an analogous manner. The increase in the excess of positive ions at the highest level is not as easily understood, though if condensation nuclei are to be used for the explanation, it might be due to the lack of water vapor.

Another explanation of the effect seems equally tenable. One assumes first that this stratification of the electricity in the air is never, under normal fine weather conditions, sufficient to reverse, to any great extent, the normal positive potential gradient. In expansion apparatus of the Wilson type it has been found that water condenses on negative ions with smaller expansions than on positive. As negative ions ascend in the atmosphere they reach temperatures and pressures at which they become condensation nuclei and so would not be included in the measurements taken in the present experiments. The result is the apparent excess of positive ions at the cloud level. Positive ions would condense higher up and so show a minimum as in the curves in Fig. 3. Whether the positive ions come from above or below does not matter as the amount of water vapor available for condensation is rapidly decreasing. In order to get sufficient data to prove or disprove these theories, potential gradient measurements would have to be taken at the same time as ionization measurement.

The cumulus clouds in the sky during Flight 4 were those which often develop into local thunderstorms or the cumulo-nimbus type. The work of Schonland (7) and Wormell (12) seems to indicate that the majority of thunderclouds are of positive polarity. That is, the centre of positive charge is above the centre of negative charge. Their conclusions were drawn from vertical current measurements on the ground during thunderstorms in England and South Africa. The present observations were taken in spaces free from visible clouds though in Flight 4 there were numerous cumulus clouds in the sky. The

process of the development of the thundercloud has never been satisfactorily explained. Assuming that the results of the present experiments indicate a layer of heavy negative ions at the cloud level and heavy positive ions higher up, in so far as the heavy ions or charged mist particles are concerned, there is a normal electrical stratification in the same direction as that found in thunderclouds. The magnitude of the charges must, of course, be negligible compared to those in a thunderstorm.

In view of the preliminary nature of these results the conclusions which have been drawn must be considered only as tentative.

A great many more observations should be made with potential gradient apparatus as well as ionization measuring equipment. The author hopes to be able to carry on this work with more elaborate equipment. With the experience gained in the present work, valuable results should be obtainable.

Acknowledgment

The author wishes to take this opportunity to thank the Aeronautics Branch of the Department of National Defence, particularly the officers who undertook the flying.

References

1. HAMSHERE, J. L. Proc. Roy. Soc. Lond. A, 127: 298-314. 1930.
2. LUGEON, J. Compt. rend. 191: 110-112. 1930.
3. MCDIARMID, Miss A. W. Phil. Mag. 6: 1132-1140. 1928.
4. NOLAN, J. J. and NEVIN, T. E. Proc. Roy. Soc. Lond. A, 127: 155-174. 1930.
5. ROSE, D. C. Can. J. Research, 5: 482-489. 1931.
6. SCHOLZ, J. Physik. Z. 32: 130-139. 1931.
7. SCHONLAND, B. F. J. Proc. Roy. Soc. Lond. A, 118: 233-251. 1928.
8. SWAN, W F. G. Terrest. Magn. 19: 81-92. 1914.
9. WIGAND, A. Ann. Physik, 66: 81-109. 1921.
10. WIGAND, A. Ann. Physik, 85: 333-361. 1928.
11. WIGAND, A. Terrest. Magn. 19: 93-101. 1914.
12. WORMELL, T. W. Proc. Roy. Soc. Lond. A, 127: 567-590. 1930.
13. ZELENY, J. Phys. Rev. 36: 35-43. 1930.

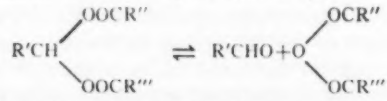
STUDIES ON HOMOGENEOUS FIRST ORDER GAS REACTIONS

I. THE DECOMPOSITION OF ETHYLIDENE DIACETATE[1]

BY C. C. COFFIN[2]

Abstract

The decomposition represented by the general equation

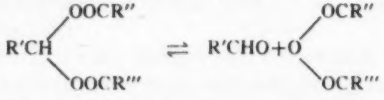


has been found to take place according to the monomolecular law. In the case of the several homologous esters already investigated at pressures above 10 cm. of mercury the reaction is entirely homogeneous, is uninfluenced by the presence of inert gases and obeys the Arrhenius equation. This paper describes the experimental method and deals with the decomposition of ethylidene diacetate to acetaldehyde and acetic anhydride at temperatures of 220° to 268° C. and at initial pressures of 11 to 46 cm. of mercury. The heat of activation is 32900 cal./mol and the velocity constants (sec^{-1}) are given by the equation, $\ln k = 23.74 - \frac{32900}{RT}$. The theoretical significance of the data is discussed.

Introduction

The theoretical significance attached to monomolecular gas reactions has recently led to the discovery of a considerable number of examples of this type of chemical change. Thus, while four or five years ago the decomposition of nitrogen pentoxide was the only generally recognized instance of such a reaction, there are now at least 14 compounds which are known to decompose by the monomolecular mechanism under certain conditions of pressure and temperature (18). However, the fact that more than half of these substances fall into either one of two classes—the aliphatic ethers investigated by Hinshelwood and his students (3, 5, 6, 7, 8, 9, 10) and the aliphatic azo compounds studied by Ramsperger (14-18)—gives an idea of the rarity of these reactions and the desirability of finding other examples with which to test current theories of molecular activation and reaction mechanism.

In the course of a systematic investigation of equilibria and reaction velocities in acid anhydride-aldehyde-ester systems from points of view outlined elsewhere (1, 2) a new series of monomolecular reactions has been found, *viz.*, the decomposition represented by the general equation



where R′, R″ and R‴ as usual represent organic radicals. The several homologous esters of this type that have already been examined in the gaseous state at pressures of 10 to 40 cm. of mercury break down homogeneously according

[1] *Manuscript received September 15, 1931.*
Contribution from the Department of Chemistry, Dalhousie University, Halifax, Canada.

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to the monomolecular law at a measurable velocity, under conditions where the equilibrium for practical purposes is shifted completely to the right. The present paper describes the experimental procedure and deals with the decomposition of ethylidene diacetate, $CH_3CH(OOCCH_3)_2$, which breaks down quantitatively to acetic anhydride and acetaldehyde. The velocity of the reaction has been determined at initial pressures of 11 to 46 cm. of mercury between the temperatures of 220 and 268° C. Rate measurements at lower pressures are being carried out at present in another apparatus as a test of Lindemann's hypothesis (13) regarding the activation mechanism of a monomolecular reaction. The decomposition of other esters of the series will be dealt with in subsequent papers.

Experimental

Apparatus and Technique

The low volatility of these esters (b.p. ethylidene diacetate = 172° C.) makes it difficult to prevent condensation, particularly at the higher pressures, in the manometer and other tubing connected with the reaction chamber, so that a considerable amount of preliminary experimentation was necessary before a satisfactory technique was developed and traces of liquid phase were completely eliminated. The method finally adopted is sufficiently different from

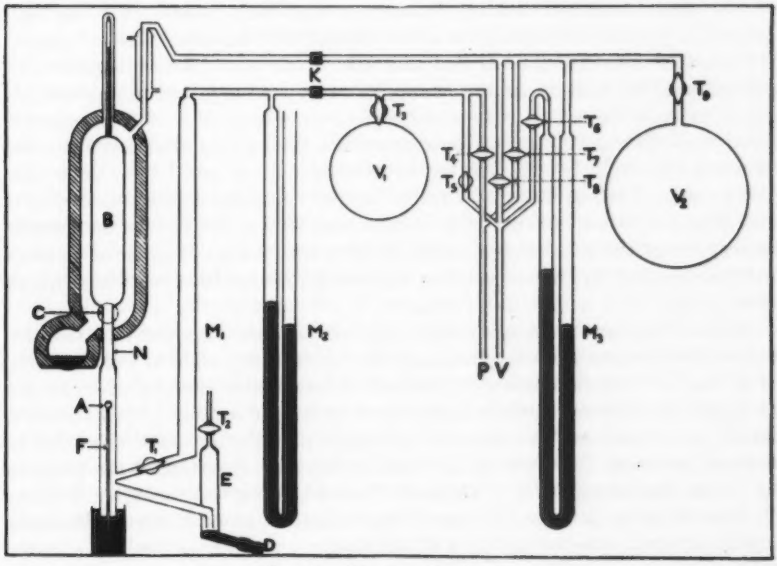

FIG. 1. *Diagram of apparatus.*

that usually employed in such work to warrant a rather detailed description. Its main disadvantage is that the reaction is carried out in the presence of mercury vapor saturated at the temperature of the reaction chamber. The

fact, however, that the logarithm of the velocity constant plotted against the reciprocal of the absolute temperature results in a straight line is evidence that for these high pressure runs at least mercury vapor is without influence on the reaction rate. This point is being studied more fully in connection with the above-mentioned low pressure decompositions.

The apparatus, which is shown in Fig. 1 (not drawn to scale), consists essentially of a reaction chamber, B (volume, 120 cc.), surrounded by the vapor of mercury boiling under a definite pressure, together with the manometers, connections to vacuum, etc., necessary for following the reaction by change of pressure at constant volume. All the apparatus to the left of the deKhotinsky seals, K, was built of Pyrex glass. The procedure in carrying out an experiment was as follows.

The mercury in the tube E was set at about the level indicated in the diagram by raising or lowering a mercury reservoir (not shown) attached to D by heavy rubber tubing which was then clamped. The rubber stopper bearing the glass tube F (closed at the bottom) was removed and a small glass bulb A almost filled at room temperature with a weighed amount of the ester was inverted in the open end of F. The rubber stopper was then replaced, surrounded by a mercury seal, and B was evacuated through P by means of two mercury condensation pumps backed by a Hyvac.

The glass jacket surrounding B was provided with a mercury boiler (gas heated), a water cooled condenser and a thermometer as shown in the diagram. All connections were glass-sealed and the whole was heavily lagged with asbestos. The pressure on the boiling mercury was adjusted to any desired value by taps T_7 and T_8 and read on the manometer M_3. V_2 represents a 12-litre stabilizing volume. The temperature of B was determined from the pressure registered on M_3, the vapor pressure data of the I.C.T. (Vol. III) being used. Throughout all the runs reported the temperature was constant to within 0.2° C. and was probably correct to at least 0.2° C. as the manometer readings were corrected to 0° C. and all the mercury used in boiler and manometers was carefully cleaned in nitric acid and distilled at least twice in a stream of air.

When B had been thoroughly evacuated and brought to a constant temperature, the clamp at D was opened and the mercury was allowed to rise slowly in M_1 and in N where it picked up the bulb A and floated it up into B. As the bulb and the mercury surface disappeared under the asbestos insulation, the tap T_1 was closed and the mercury rose rapidly in the left hand side until it reached the mark C, etched on the neck of the bulb B, where it was stopped by closing the clamp at D. The point C could be observed through a window (indicated by the dotted circle) cut in the insulation on both sides of the tube and illuminated from behind by a 60-watt lamp. As soon as the bulb of ester exploded (generally two or three seconds after reaching C) the stop clock was started and the mercury in M_1 was brought level with that at C by opening T_1 and by manipulating the clamp at D and the taps T_4 and T_5. As no attempt was made to determine the initial pressure in B the latter operation could be

carried out at leisure. At this point the stabilizing volume V_1, which was closed during the evacuation of B, was connected to the system to facilitate pressure adjustments.

The reaction was followed at constant volume by balancing the manometer C-M_1 (also illuminated from behind) and reading the pressure on the manometer M_2 at measured intervals of time. Fine adjustment of the amount of mercury in C-M_1 was accomplished by means of subsidiary screw clamps on the rubber tube at D, while an accurate balance was obtained by the use of a sighting level consisting of a long straight 3-cm. glass tube almost filled with water, and adjustably fixed in a horizontal position about one foot from the manometer. This tube, adjusted until the air bubble remained in its middle, served as a convenient reference in balancing the manometer. M_2 and M_3 were backed by calibrated mirror scales graduated in millimetres.

To obtain the true pressure of the reaction, the vapor pressure of mercury at the temperature of B must of course be subtracted from all pressures registered on M_2. That the mercury surface at C exerts a pressure equal to that in the vapor jacket, in spite of the chance for heat leak through the mercury column below it, was confirmed by an experiment in which the vapor pressure of mercury was determined from 220° to 280° C. With B completely evacuated and the mercury surface held at C the readings of manometers M_2 and M_3 agreed within 1 mm. over the temperature range investigated. This pressure was attained within about two minutes of raising a cold mercury surface to C.

After a run was over all the mercury to the left of the clamp D was removed from the apparatus in order to avoid contamination of the next experiment by entrained reaction products, the cold tubing below C was wiped free of liquid and broken glass with clean cotton swabs, and the whole apparatus was thoroughly evacuated and flushed out several times with dry air. The reaction chamber was kept hot and the tubing below it was heated with a smoky flame during evacuation. When the tubing had cooled a fresh bulb of ester was introduced, the apparatus was again evacuated and the next run started.

As is pointed out in a later section, the nature of the formula by which the rate constant is calculated makes it necessary to determine the initial pressure with considerable accuracy if uniform constants are to be obtained over the whole run. It being impossible to determine the initial pressure directly with any certainty, recourse was had to extrapolation which in general gave quite satisfactory results. Due however to the fact that some uncertainty also existed in determining zero time, giving rise to a like uncertainty in the extrapolated initial pressure, more confidence is placed in the results calculated from the final pressure which is assumed to be twice the initial pressure. The final pressure had also to be determined by extrapolation since on account of a very slow secondary reaction, presumably the decomposition of acetaldehyde (9), the pressure never reached a stationary value. Each run was therefore continued for at least 24 hr. (at the lower temperatures for 48 hr.) and the true final pressure obtained by extrapolating the linear part of the pressure-time curve back to zero time. (By closing T_1 the apparatus could be left to itself

for an indefinite period.) This curve always straightened out after about 300 to 500 min. depending on the temperature and, at 268°C., had a slope equivalent to a pressure change of about 0.01 mm./min. The slope at the lower temperatures was much smaller giving a still more trustworthy extrapolation.

The velocity constants calculated from the initial pressure obtained in this way showed no drift even when the reaction was 90 to 95% complete, while those calculated from the directly extrapolated values of the initial pressure almost invariably showed a drift up or down toward the end of the reaction, depending on whether too high or too low a value was taken for the pressure at zero time. Constants calculated from initial pressures determined from the weight of the ester by the ideal gas laws were still more unsatisfactory, presumably on account of the large deviation of such a system from that of an ideal gas. All constants were calculated by a graphical method as explained in a later section.

Purification of the Ester

The ethylidene diacetate was obtained through the courtesy of the Shawinigan Laboratories Ltd.* It contained about 2% of acetic acid which was removed by washing with an excess of 0.3 *N* baryta, drying with $CaCl_2$ and fractionating twice *in vacuo*. The middle third, which was used in these experiments, analyzed 0.083% acetic acid and contained no other probable impurity such as acetic anhydride, vinyl acetate or acetaldehyde. It melted sharply at 17.5° C.

Products of the Reaction

As preliminary experiments had indicated the decomposition to be practically free from complicating side reactions, no attempt was made to analyze the contents of the reaction chamber *B* after a run. Instead a series of experiments was carried out in which the ester was passed through two metres of 5-mm. Pyrex tubing, in the form of a coil, enclosed in a piece of 2-in. iron pipe wound with nichrome wire. Temperatures were measured with a copper-constantan thermocouple in the centre of the furnace which was regulated by hand. Some 200 gm. of the ester was slowly introduced from a burette at as uniform a rate as possible, while the products were condensed in a CO_2-ether mixture and analyzed for acetaldehyde, acetic anhydride, acetic acid, vinyl acetate and ethylidene diacetate by standard methods.

Below about 250° C. the only products were acetaldehyde and acetic anhydride which were found in approximately equimolecular proportions and which corresponded, within the limits of error of the rather difficult analysis, to the amount of ester disappearing. The amount of the latter decomposed in any experiment depended of course on the temperature of the furnace and the rate of flow through the tube. No attempt was made to determine reaction velocities or equilibria by this dynamic method.

Above 300° C. a small quantity of acetic acid appeared which increased as

*The author wishes to take this opportunity of expressing his thanks to the Shawinigan Laboratories Ltd., and particularly to Mr. W. R. Elliot of that organization for the gift of two kilograms of purified ethylidene diacetate together with its analyses and particulars relating to its history and physical properties.

the temperature was raised until at 400° C. it amounted to about 10 molecules per 100 molecules of ester decomposed. The acetic anhydride and acetaldehyde (plus its decomposition products) still being present in equimolecular proportions, it is concluded that the acid instead of being a product of the hydration from some source of the anhydride comes directly from the ester itself according to the equation,

$$CH_3CH(OOCCH_3)_2 \rightleftarrows CH_3COOH + CH_2{=}CHOOCCH_3.$$

A trace of vinyl acetate invariably accompanying the acetic acid lends support to this hypothesis. The fact that the vinyl acetate is never equivalent to the acetic acid is probably due to its immediate polymerization at the high temperature of the reaction tube. This would also account for the fact that the walls of the Pyrex coil became coated with a charred deposit during a high temperature run.

In the experiments above 350° C. a small quantity of permanent gas was also produced. This, collected over water and analyzed, was found to be CO and CH_4 in approximately equivalent quantities and is thus in all probability the result of some acetaldehyde decomposition (9).

Since in these dynamic experiments no product other than acetic anhydride and acetaldehyde was found below 300° C. it may be assumed that these are the only products of the static decompositions as well. That the equilibrium is shifted far enough to the right to make unnecessary any correction in the ordinary monomolecular equation for the velocity of the reverse reaction, is proved by the fact that the final pressure obtained by extrapolating, as described above, is always twice the extrapolated initial pressure within the limit of error of the later determination.

Since submitting the manuscript of this paper for publication the writer's attention has been called to a recent observation by Kassel (12) on the accelerated decomposition of acetaldehyde in the presence of mercury vapor. As this effect was observed to be measurable at temperatures as low as 177° C. the possibility is at once suggested that the true course of the ester decompositions described in this paper cannot be determined by pressure changes. In order to show that such a possibility is not a probability it is necessary to refer to several experiments which were carried out during the course of the present work and in which the effects mentioned by Kassel were not observed. Acetaldehyde vapor at pressures of from 10 to 30 cm. of mercury was admitted to the reaction chamber of the apparatus described above, the mercury was raised to the mark *C* and pressure readings were made from time to time over a period, in one case, of three days. In none of these experiments, all of which were carried out at 263° C., was the rate of pressure increase greater than that involved in the above-mentioned extrapolation, so that, except for a small uncertainty with regard to the final pressure, complications arising from the decomposition of acetaldehyde appear to be negligible.

Results

Calculation of Velocity Constants, etc.

The reaction being of the type $A \rightleftarrows B + C$ the partial pressure of the ester (A)

at time t is equal to $2Po - P$, where Po is the total pressure at the beginning and P is the total pressure at time t, so that, under conditions where the equilibrium is shifted completely to the right, the monomolecular and bimolecular equations take the respective forms,

$$k_1 = \frac{1}{t} 2.303 \log \frac{Po}{2Po - P}$$

$$k_2 = \frac{1}{t} \frac{P - Po}{Po(2Po - P)},$$

i.e., if the graph of the expression $\frac{P - Po}{Po(2Po - P)}$ against time is a straight line the reaction is bimolecular, while if that of $\log \frac{Po}{2Po - P}$ against time gives a straight line the reaction is monomolecular. In Fig. 2 $\log \frac{Po}{2Po - P}$ is plotted against time for Runs 12 to 25 of Table I, which is a summary of all the experiments made to date in the apparatus described above. It is evident from the straight lines obtained that the reaction is definitely monomolecular with constants (sec^{-1})

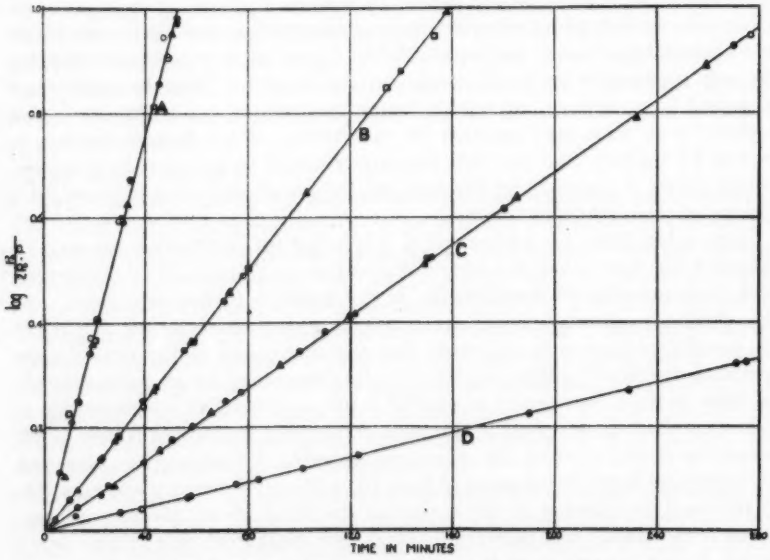

FIG. 2. *Plot of log* $\frac{Po}{2Po-P}$ *against time (Runs 12-25).*

at the different temperatures equal to the slopes of the lines multiplied by $\frac{2.303}{60}$. The expression $\frac{P - Po}{Po(2Po - P)}$ plotted against time invariably gives a very pronounced curve.

In Runs 3 to 11 (Table I) the initial pressure was determined directly by extrapolation while Runs 12 to 25 were carried on for a time long enough to

extrapolate for the final pressure which was taken to be twice the initial pressure. For typographical reasons the data of the last set of runs only are plotted in Fig. 2. The first runs give results quite as good, until the reaction is about 80% complete, when some drift usually occurs on account of the above-mentioned uncertainty in the extrapolation. In each case the results were plotted on a large scale (x axis; 1 min. = 2.5 mm.: y axis; 1.0 = 500 mm.), the best straight line was drawn through the points obtained for all the runs at any one temperature and the rate constant was determined from the slope of the line. More weight was given to the points taken in the first half than to those in the last half of the reaction, on account of the magnification of the effect of the error in Po as $2Po-P$ decreases. The constants at the different temperatures are given in column 6 of Table I. They are considered to be correct to within at least 3%.

The fact that the time to half-value ($\log \frac{Po}{2Po-P} = 0.3$) at any temperature is independent of the initial pressure is well shown in Fig. 2 and affords conclusive proof of the strictly monomolecular character of the reaction. For example the weights of ester used in Runs 16 to 20 (Fig. 2, Curve A) were

TABLE I

EXPERIMENTAL RESULTS

Run No.	T °Abs.	Weight of ester, gm.	Po cm. Hg.	$\frac{Po}{\text{Wt. of ester}}$	k sec^{-1}	
3	515.0	0.1980	36.2	183		
4	515.0	0.2049	38.0	185	2.05×10^{-4}	
5	515.0	0.1036	19.9	191		
6	515.0	0.1083	21.0	194		
7	540.0	0.2192	44.3	202		
8	540.0	0.1747	35.8	205	9.14×10^{-4}	
9	540.0	0.1846	37.5	203		
10	529.0	0.2399	47.0	196	4.80×10^{-4}	
11	529.0	0.1343	26.3	196		
12	517.9	0.1364	35.00	256		Curve B○*
13	517.9	0.1578	40.15	254	2.41×10^{-4}	Curve B⊕*
14	517.9	0.1686	31.61	187		Curve B△
15	517.9	0.1660	31.10	187		Curve B□
16	535.8	0.1805	35.64	197		Curve A○
17	535.8	0.1881	36.55	194		Curve A⊕
18	535.8	0.0570	11.51	202	7.24×10^{-4}	Curve A△
19	535.8	0.0817	16.83	205		Curve A□
20	535.8	0.1312	26.20	200		Curve A⊖
21	507.4	0.1244	22.32	179		Curve C○
22	507.4	0.2616	46.28	177	1.32×10^{-4}	Curve C⊕
23	507.4	0.1101	19.80	179		Curve C△
24	493.0	0.2183	37.70	169	4.54×10^{-5}	Curve D○
25	493.0	0.2438	41.82	171		Curve D⊕

*NOTE:—*Surface-volume ratio increased about 20 times.*

varied from 0.057 to 0.188 gm. without bringing to light any appreciable trend in the velocity constant. It is to be noted that at $\log \frac{Po}{2Po-P} = 1.0$ the reaction is 90% complete.

As has already been mentioned it was found necessary to determine zero time by back extrapolation of the straight line obtained by plotting $\log \frac{Po}{2Po-P}$ against time. The zero so estimated was generally four to eight minutes after the bursting of the bulb and thus suggests the existence of an "induction period". This point is under investigation at present. The slope of the line, *i.e.*, the rate constant, is uninfluenced by this uncertainty with regard to zero time.

Column 5 gives the values of the ratio *Po*/weight ester. If the equilibrium were not shifted completely to the right this ratio should increase with decreasing pressure. A slight difference is in fact usually to be observed in the runs at any one temperature (*e.g.*, Runs 16 to 20) but as it is of the order of magnitude to be expected from deviations from the ideal gas laws it is probable that even at the higher pressures the ester is completely dissociated. On account of the small heat of reaction the equilibrium constant would not be expected to change very much with temperature.

Homogeneity of the Reaction

In Runs 12 and 13 the surface-volume ratio was increased about 20 times by filling *B* with short lengths of small Pyrex tubing held in place by a plug of glass wool above *C*. From Fig. 2 (Curve B Run 12, ○, Run 15, ⊕) it is evident that the velocity of the reaction is absolutely independent of the extent of the glass surface of the reaction chamber, *i.e.*, the reaction takes place homogeneously throughout the body of the gas.

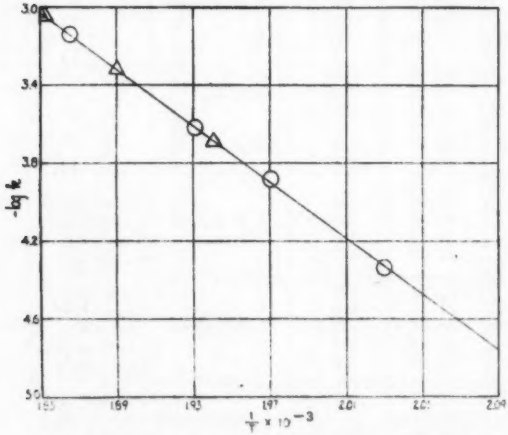

FIG. 3. *Graph of log k against 1/T. The circles represent the data of Runs 12 to 25 and the triangles those of Runs 3 to 11.*

After a few runs at the higher temperatures the walls of *B* become coated with a very thin brown deposit which can be washed off with alcohol and ether as thin flakes of film resembling collodion. This is probably polymerized vinyl acetate liberated with acetic acid in the secondary reaction already discussed. Between Runs 18 and 19 the apparatus was taken down and *B* was thoroughly cleaned with alcohol, ether and hot chromic acid solution. No difference in the velocity of the runs before and after cleaning could be detected.

The Energy of Activation

Fig. 3 gives the usual graph of $\log k$ against $\frac{1}{T}$. The straight line obtained shows that the reaction obeys the Arrhenius equation and gives a heat of activation of 32,900 cal./mol. The circles represent the data of Runs 12 to 25 and the triangles those of Runs 3 to 11. The velocity constant at any temperature is given by the equation,

$$\ln k = 23.74 - \frac{32900}{RT}.$$

The energy of activation and the absolute temperature at which the decomposition attains a given rate, agree well with the parallelism exhibited by the known examples of first order reactions (5).

Discussion

The fact that these esters decompose by the monomolecular mechanism is in harmony with the suggestion of Hinshelwood (7) and others, that first order reactions are characteristic of complicated molecules which can use their large number of internal degrees of freedom as energy reservoirs, and so be temporarily independent of activating collisions in the sense that the latter are necessary for bimolecular reactions. Moreover although the decomposition is endothermic the heat of reaction is small, and hence from thermochemical considerations a large heat of activation is not required (5). A monomolecular decomposition above a certain limiting pressure is therefore to be expected.

The generally accepted picture of the monomolecular mechanism in the gaseous state is based on the suggestion of Lindemann (13) that there exists a time lag between activation and reaction, which is large compared to the time between molecular collisions, so that the majority of molecules activated at any instant suffers deactivation and only a small constant fraction succeeds in reacting. In other words, the essentially bimolecular processes of collisional activation and deactivation take place at a rate greater than that at which activated molecules are lost by reaction. The fraction of molecules activated at any instant being thus in excess of the fraction reacting at that instant and independent of the pressure of the reactant, the reaction velocity is also independent of the pressure of the reactant and kinetically the decomposition follows the monomolecular law. The maintenance of this excess of activated molecules is therefore a result of processes characteristic of pressures at which the "mean free time" is small compared to the average time between activation and reaction, and it is to be expected that as the pressure is lowered a point will be reached where these two time intervals approach equality, with the result that an increasing fraction of activated molecules will be removed by reaction and the velocity constant will begin to fall. That this theory gives essentially the correct picture is evinced by the fact that the majority of the known monomolecular reactions do show just such a decrease in velocity as the pressure is lowered beyond a certain value.

This limiting pressure is a specific property of the molecule in question, being dependent on the magnitude of the time lag between activation and reaction

or upon the number of internal degrees of freedom involved in the activation process. At present the scarcity of data concerning monomolecular reactions allows little to be predicted with regard to this number, beyond the fact that it apparently increases with the complexity of the reaction and the molecule. Highly specific structural effects have been observed (3) but as yet the data are insufficient to warrant any generalizations whatsoever.

It is thus impossible to say whether or not the esters of this series possess too complicated an activation mechanism to show the predicted decrease in velocity constant at measurable pressures. If this decrease, which is now being looked for, does occur before the pressure becomes too small to measure, it is possible that this series of reactions will furnish interesting data with regard to the effect of molecular structure on the number of internal degrees of freedom associated with the activation process. Such data are particularly desirable in view of the fact that the majority of the monomolecular reactions described in the literature are more or less complex "pyrolytic" decompositions which involve the rupture of several bonds at once, are apt to undergo considerable changes in mechanism with alterations in molecular structure and which in general are too complicated to furnish much information with regard to the details of activation or reaction. The comparative simplicity, therefore, of the decomposition of these esters, together with the fact that the reaction mechanism appears to remain unchanged throughout the series, are important considerations in connection with the theoretical significance of the velocity data.

The system being of the type $A \rightleftarrows B+C$ the existence of a homogeneous reverse reaction and the reality of a statistical equilibrium in the gaseous state are of particular interest in connection with the "dreierstoss" theory. Since Herzfeld (4) first pointed out that the reaction of two atoms to form a molecule must be a termolecular or a wall reaction in order that the freshly formed molecule be deactivated and immediate decomposition prevented, a tendency has existed to extend this idea to include all bimolecular association reactions. In a recent discussion of the possibility of such reactions Kassel (11) has argued that, although none of the monomolecular reactions so far investigated lead to a measurable equilibrium, the existence of the time lag demanded by Lindemann's hypothesis between activation and reaction removes such cases from the restriction of the "dreierstoss" theory. He states, "A complex molecule once formed at a binary collision may then be stabilized at any subsequent collision within this period (of time)."

While it is not quite clear how such a molecule can be formed in the first place, since at the moment of incipient formation it would be expected to possess just that energy distribution which ordinarily occurs only after the lapse of the time interval in question, it nevertheless seems probable from Kassel's arguments that such reactions can and do occur. It is evident, however, that under the conditions of the foregoing experiments the velocity of the reaction between acetaldehyde and acetic anhydride is, like the reverse of other first order changes, negligible in comparison with that of the ester decom-

position. Whether or not other conditions of pressure and temperature will bring about a measurable equilibrium and enable the kinetics of the reverse reaction to be examined remains to be seen. This point, which is of interest also in connection with the readily determined liquid phase equilibrium, is being investigated.

References

1. COFFIN, C. C. and MAASS, O. Can. J. Research, 3: 526-539. 1930.
2. COFFIN, C. C. and MAASS, O. Can. J. Research, 3: 540-542. 1930.
3. GLASS, J. V. S. and HINSHELWOOD, C. N. J. Chem. Soc. 1804-1814. 1929.
4. HERZFELD, K. F. Z. Physik, 8: 132-136. 1922.
5. HINSHELWOOD, C. N. Kinetics of chemical change in gaseous systems. 2d. ed. Oxford. 1929.
6. HINSHELWOOD, C. N. Proc. Roy. Soc. Lond. A, 113: 230-233. 1927.
7. HINSHELWOOD, C. N. Proc. Roy. Soc. Lond. A, 114: 84-97. 1927.
8. HINSHELWOOD, C. N. and ASKEY, P. J. Proc. Roy. Soc. Lond. A, 115: 215-226. 1927.
9. HINSHELWOOD, C. N. and HUTCHISON, W. K. Proc. Roy. Soc. Lond. A, 111: 380-385. 1926.
10. HINSHELWOOD, C. N. and THOMPSON, H. W. Proc. Roy. Soc. Lond. A, 113: 221-229. 1927.
11. KASSEL, L. S. J. Am. Chem. Soc. 53: 2143-2147. 1931.
12. KASSEL, L. S. J. Phys. Chem. 34: 1166-1173. 1930.
13. LINDEMANN, F. A. Trans. Faraday Soc. 17: 598-599. 1922.
14. RAMSPERGER, H. C. J. Am. Chem Soc. 49: 912-916. 1927.
15. RAMSPERGER, H. C. J. Am. Chem. Soc. 49: 1495-1499. 1927.
16. RAMSPERGER, H. C. J. Am. Chem. Soc. 50: 714-721. 1928.
17. RAMSPERGER, H. C. J. Am. Chem. Soc. 51: 2134-2143. 1929.
18. RAMSPERGER, H. C. and LEERMAKERS, J. A. J. Am. Chem. Soc. 53: 2061-2071. 1931.

THE OXIDATION OF ACETALDEHYDE[1]

By W. H. Hatcher[2], E. W. R. Steacie[3] and F. Howland[4]

Abstract

Acetaldehyde, when freshly distilled, suffers immediate oxidation on coming into contact with oxygen or air. A compound is produced which in aqueous solution behaves as an organic peracid. This has a pronounced effect upon the subsequent gas-phase oxidation of the acetaldehyde.

Introduction

During a study of the oxidation of acetaldehyde in the vapor phase, the obviation of certain difficulties which were encountered has uncovered the susceptibility of this compound to attack by oxygen, even at room temperature. In this communication it is not proposed to discuss the results of the systematic study of the gas-phase oxidation of acetaldehyde but merely to touch on those results which bear on the susceptibility of the compound to low temperature oxidation.

Bowen and Tietz (1) have recently stated that acetaldehyde and oxygen in ultra-violet light produce peracetic acid. It has long been known that benzaldehyde behaves similarly, but in the past insufficient attention has been paid to such behavior on the part of aliphatic aldehydes. It has also been observed, during the course of measurements on the rate of polymerization of acetaldehyde (2), that a serious change in the velocity of polymerization occurs if the material is exposed to the air. The following pages will serve to emphasize the necessity of the absolute exclusion of air or oxygen from acetaldehyde during its preparation for subsequent experimental work.

Experimental

The course of the high temperature oxidation of acetaldehyde was followed by the rate of change in pressure of a mixture of acetaldehyde and oxygen in a system which permitted but a negligible change in volume. The gases were first mixed in a bulb and were then let into the reaction flask, where the pressure was read by means of a capillary mercury manometer.

The oxygen used was obtained from a cylinder and was not purified except by drying over phosphorus pentoxide. The acetaldehyde was prepared from high grade paraldehyde by distillation with a few drops of concentrated sulphuric acid.

Preliminary experiments were carried out from 175 to 250°C. with partial pressures of acetaldehyde from 3 to 12 cm., together with one to three times as much oxygen. It was found that at these temperatures the reaction was accompanied at first by a decrease in the total pressure. After reaching a minimum the pressure then rose again and a constant value was finally reached

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which was considerably higher than the initial pressure. In these experiments excess oxygen was always used.

In these preliminary experiments it was not possible to obtain results for the rate of oxidation which were at all consistent. In addition, explosions sometimes occurred a few seconds after the mixture of gases reached the reaction flask. At the same time difficulty was encountered in keeping the reaction flask, as well as the tubing leading to it, free from a white crystalline deposit. On examination this deposit proved to be a compound of mercury, since it yielded mercury on decomposition.

Several cc. of mercury were therefore introduced into the reaction flask and oxidation experiments were carried out at 120° C. After each experiment a considerable quantity of this white material was found on the mercury surface. A large quantity of the material was then prepared under similar conditions. It seemed probable that it should be a mercurous or mercuric salt of either formic or acetic acid. A comparison of many properties proved that the substance in question was mercurous acetate.

The apparatus was therefore modified and the reactants were prevented from coming into contact with mercury by means of an air buffer above the manometer surface. Thereafter no deposit formed in the tubing, and explosions never occurred.

After the above changes, however, it was still impossible to obtain consistent results. Examination of the results indicated that the velocity of the oxidation and the total pressure drop were apparently decreased by allowing the mixture of oxygen and acetaldehyde to stand for any appreciable length of time in the mixing bulb before being sent into the reaction flask.

For example, at 118° C. with a partial pressure of acetaldehyde of 13.7 cm., and a partial oxygen pressure of 20.5 cm., the maximum diminution in pressure was 92% of the partial pressure of acetaldehyde if the reactants were admitted to the bulb immediately after mixing. If, however, the reactants were allowed to stand for 30 min. before admission to the reaction flask the total pressure drop was only 83% of the initial partial pressure of acetaldehyde. If the reactants stood for two hours before admission the pressure drop diminished still further to 69% of the initial pressure of acetaldehyde.

These discrepancies can be explained if the oxidation of acetaldehyde proceeds to a marked extent at room temperature. If this were so the calculated value for the initial pressure of acetaldehyde would be too high. The calculated pressure drop, expressed in terms of acetaldehyde pressure, would therefore be too low as is observed experimentally.

It seemed reasonable to assume that if as short a contact period for acetaldehyde and oxygen as 30 min. could have such a large effect, the oxidation of acetaldehyde might be appreciable even during its distillation, or when it came into contact with air for a brief period. This assumption is borne out by the fact that Bowen and Tietz (1) found that liquid acetaldehyde absorbs oxygen quite rapidly at room temperature. In the gas-phase oxidation the maximum pressure drop encountered in the initial phase of the reaction was never

greater than the initial partial pressure of acetaldehyde. This would indicate that the reaction occurring is

$$CH_3CHO + O_2 \rightleftharpoons (CH_3CHO.O_2),$$

and that some oxide or peroxide of acetaldehyde is formed. Such a compound would be unstable and would give an explanation of the explosions encountered when acetaldehyde-oxygen mixtures came into contact with mercury.

In view of these conclusions a few drops of acetaldehyde which had been freshly distilled in a thoroughly cleaned glass apparatus were dropped into a neutral 10% solution of potassium iodide. Iodine was liberated at once in sufficient quantity to produce a deep yellowish-brown color. This reaction, a test for a peroxygen compound, was obtained immediately after the distillation of acetaldehyde, as well as several hours later. A mixture of acetaldehyde vapor and oxygen gave the same result when shaken with a solution of potassium iodide.

In order to obtain acetaldehyde free from a trace of any peroxygen compound it was necessary to distil it in the absence of air and to keep it out of contact with air thereafter. Acetaldehyde was therefore distilled in the presence of carbon dioxide. In these distillations dry, oxygen-free, carbon dioxide was circulated through an air-tight distillation apparatus for two hours. Acetaldehyde was then run into the distilling flask from a dropping funnel. Potassium iodide solution which had been boiled to remove dissolved oxygen was simultaneously run into the ice-cooled receiver of the distillation apparatus. Twenty drops of acetaldehyde dropping directly from the condenser failed to produce any color change in the solution of potassium iodide. If, however, air was momentarily let into the apparatus, the next five drops of acetaldehyde produced a deep yellow color. Apparently then, a momentary contact with air is sufficient to produce an appreciable quantity of some peroxygen compound.

In a further experiment oxygen was bubbled through a colorless solution of potassium iodide and acetaldehyde which was kept cold in an ice bath. After 30 min. of such treatment a slight yellow color was detected, which deepened somewhat when the solution was warmed to room temperature. However, the color never approximated in depth that produced by five drops of acetaldehyde which had come into contact with air before reaching the solution.

The instantaneous liberation of iodine from a neutral solution of potassium iodide is, of course, characteristic of a so-called peracid. On the other hand, an organic peroxide requires time to hydrolyze to the peracid before any coloration becomes apparent. The acetaldehyde-oxygen complex would therefore seem to possess the characteristics of an organic peracid.

It is not difficult to formulate a mechanism for the above reactions, but it is deemed advisable to defer this until the completion of the investigation of the gas-phase oxidation. In addition, investigations are under way on the properties of oxygen-free acetaldehyde.

References

1. BOWEN, E. J. and TIETZ, E. L. Nature 124: 914. 1929.
2. HATCHER, W. H. and BRODIE, B. Can. J. Research, 4: 574-581. 1931.

THE ALKALOIDS OF *SENECIO* SPECIES

I. THE NECINES AND NECIC ACIDS FROM *S. RETRORSUS* AND *S. JACOBAEA*[1]

By Richard H. F. Manske[2]

Abstract

A new alkaloid, *retrorsine*, has been isolated from *Senecio retrorsus* of South African origin. Analysis of the free base and its methiodide together with its hydrolytic products point to the empirical formula, $C_{18}H_{25}O_6N$. Hydrolysis yields a new base, $C_8H_{13}O_2N$, termed *retronecine* together with an acid, *retronecic acid*, isolated as the monolactone, $C_{10}H_{14}O_5$. The base is tertiary, contains one hydroxyl (benzoyl derivative) and probably a ketonic group. The preparation of the di-*p*-phenylphenacyl derivative of the acid proves its dibasicity. The alkaloid, jacobine, from *S. jacobaea* has been isolated in a state of purity and probably has the empirical formula $C_{18}H_{25}O_6N$, although this is only partly confirmed by analysis of the hydrolytic fragments. The necine derived from jacobine is shown to be identical with retronecine.

An examination of *S. aureus* failed to show the presence of an alkaloid in tractable amounts. A system of simplified nomenclature to designate the *Senecio* alkaloids and their hydrolytic products is suggested.

The *Senecio* species belong to the natural order Compositae and enjoy the distinction of being the only members of this order which have been shown to contain well-defined alkaloids in appreciable quantities. It frequently occurs that different species belonging to the same botanical genus elaborate a group of alkaloids of similar if not identical structure although the associated minor alkaloids may vary considerably. As an example the cinchona alkaloids serve well, in that they are confined entirely to the suborder Cinchonoideae of the N.O. Rubiaceae which is particularly rich in alkaloids, and each suborder is characterized by its own group of closely related bases. In the N.O. Ranunculaceae the occurrence of the characteristic aconitines is confined exclusively to the genus *Aconitum*, and no species of this genus has been found to be devoid of alkaloids. On the other hand some alkaloids seem to represent the final stages of a diversity of phytochemical processes and no better example than berberine, which occurs in five different natural orders, could be cited.

Several species of *Senecio*, *e.g.*, *S. kämpferi* (4), appear to be devoid of alkaloids and an examination of *S. aureus* has now shown that if any is present the amount is excessively small.

It seemed of interest therefore to investigate in some detail a number of *Senecio* species and to ascertain whether or not the alkaloids from the various sources are closely related structurally, if indeed not identical in some cases. Added interest is attached to the investigation because of the fact that the alkaloids in question appear to be rather toxic and produce cumulative effects when ingested by livestock in small doses over extended periods. Winton disease, so common in the Maritime Provinces, has been shown to be caused by the Giant Ragwart (*S. jacobaea*) and a similar malady due to *S. latifolius* and to *S. burchelli* has been responsible for many losses among livestock in

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South Africa. The common groundsel (*S. vulgaris*) of European origin is not known to be toxic although it was the first to be examined chemically. From the latter, Grandval and Lajoux (3) isolated two alkaloids in small amounts. Only one, however, was obtained in sufficient quantity for analysis.

The only other contribution to our knowledge of *Senecio* alkaloids which deserves mention is that of Watt (5) dealing with *S. latifolius*. Two well-defined alkaloids, senecifoline ($C_{18}H_{27}O_6N$) and senecifolidine ($C_{18}H_{25}O_7N$) were shown to be present, the latter in traces only. Senecifoline on treatment with potassium hydroxide in absolute alcohol was readily hydrolyzed to an acidic and a basic fragment. The latter, termed senecifolinine, was isolated as the hydrochloride (m.p. 168° C.) and on the basis of analysis was given the formula $C_8H_{11}O_2N$. The acid (m.p. 198° C.), termed senecifolic acid, as the result of combustion and titration, was given the formula $C_{10}H_{16}O_6$, and was assumed to be a dibasic acid. Watt pointed out that the elements of a molecule of water were not taken up in this hydrolysis, a point which was admittedly obscure.

Owing to the possible complexity involved in naming the parent alkaloids together with the hydrolytic products of each, it seems desirable to simplify the nomenclature somewhat, and it is proposed to coin the generic name, *necine*, for the basic hydrolytic product and to reserve the name, *necic* acid, for the acidic fragment.

The present memoir contains an account of a preliminary examination of the alkaloids of *S. retrorsus* and of *S. jacobaea*. The former plant material was of South African origin and the author wishes to express his indebtedness to Dr. E. Percy Phillips, Principal Botanist of the Union of South Africa (Pretoria) who generously supplied the material. There appears to be some doubt regarding the specific name owing to the uncertainty and confusion surrounding the identification and naming of the *Senecio* species of South African habitat, and until this point has been clearly settled the exact botanical origin must be left undecided. The choice, however, is particularly fortunate on account of the comparatively high content of alkaloid and furthermore the material was probably homogeneous since a thorough search failed to reveal more than one base.

The alkaloid crystallizes with great facility from a variety of solvents and in common with the others derived from *Senecio* species has no sharp melting point. The most characteristic derivative is the methiodide which crystallizes from hot water in stout brilliant prisms. Analytical figures of the base and of the methiodide are in good agreement with the formula $C_{18}H_{25}O_6N$, which is amply confirmed by analysis of the hydrolytic products. Inasmuch as the substance appears to be new the name *retrorsine* is proposed for it.

Hydrolysis yielded the necine, $C_8H_{13}O_2N$, isolated as the hydrochloride, for which the name *retronecine* is suggested, together with an acid, $C_{10}H_{16}O_6$, now termed *retronecic* acid. Retronecine hydrochloride crystallizes in deep tetragonal plates melting at 164° C.* and on the basic of its melting point and formula appears to differ from Watt's senecifolinine hydrochloride. Benzoylation with excess benzoyl chloride in the presence of potassium carbonate yields

*All melting points are corrected.

only a monobenzoyl derivative which is still basic and yields a homogeneous methiodide. It therefore appears that the nitrogen is tertiary and that one, and only one, oxygen is present as a hydroxyl group. Some evidence that the remaining oxygen atom is present as a ketonic group is found in the fact that on treatment with piperonal and alkali an intense yellow color is developed. This observation further indicates that the groups $—CH_2.CO—$, or $—CH_2.CO.CH_2—$ may be present.

Retronecic acid forms a dipotassium salt which is sparingly soluble in absolute alcohol and crystallizes almost quantitatively from the reaction mixture during hydrolysis. The potassium salt on treatment with *p*-phenylphenacyl bromide (2) yields the corresponding diphenacyl derivative, an observation which sufficiently proves the acid to be dibasic. It is, however, not likely that the two carboxyl groups are concerned in the esterification of retronecine to form the parent alkaloid. Such a supposition would require two hydroxyls in retronecine which are probably not there, and the presence of at least an eight membered ring containing two ester groups,—an unlikely constitution. The latter supposition furthermore is not consistent with the observation that only one molecule of water is taken up in the hydrolysis of retrorsine. There remains the logical alternative therefore that only one hydroxyl is concerned in the esterification and that the remaining one functions as a lactone. This view is largely confirmed by the observation that retronecic acid loses a molecule of water with extreme ease. It suffices to heat the acid for a short time on the water bath or to evaporate its solution in ethyl acetate and the resulting product, almost certainly the monolactone, crystallizes readily.

Although it has been known for some time that one or more alkaloids are present in *S. jacobaea* (1) the base or bases have not been satisfactorily characterized. It has now been possible to examine the base in some detail due to the courtesy and valued co-operation of Mr. R. R. Hurst, Plant Pathologist in charge at Charlottetown, P.E.I., who on numerous occasions supplied material of authentic botanical origin.

The alkaloid crystallizes with great facility but unfortunately the total content of the plant is exceedingly low. Analysis of the base and its methiodide point to the expirical formula $C_{18}H_{23}O_5N$, and this is partly confirmed by analysis of the hydrolytic products. The name, *jacobine*, indicative of its botanical origin is proposed for the alkaloid.

The necine obtained by the procedure already referred to is identical with retronecine but the necic acid, for which the name *jaconecic* acid is coined is different from retronecic acid and furthermore does not appear to be identical with senecifolic acid (5). Analyses give values in substantial agreement with the formula $C_{10}H_{16}O_6$, *i.e.*, the same as senecifolic acid. This formula is given with reserve and it is proposed to continue the investigation when more material is available.

During the isolation of the alkaloids from the above two species a number of other products were encountered, an examination of which is reserved for a later opportunity. Two substances, however, may be mentioned in passing.

The aqueous acid solution obtained from *S. retrosus* yielded to chloroform extraction a mixture from which one substance crystallized with great facility in large colorless hexagonal plates. Except for the analytical data which indicate $C_{12}H_{16}O_7$, its properties have not been investigated.

The second substance has been obtained only from *S. jacobaea* as a phenylhydrazone, melting at 178° C. Analytical figures are in good agreement with $C_7H_6O_2 : N.NH.C_6H_5$, and although the melting point of protocatechuic aldehyde phenylhydrazone is also close to 178° C. (6), a mixture of the two begins to sinter some 20° C. lower. The substance, prior to combination with phenylhydrazine, is not extracted appreciably from aqueous solution by chloroform.

Experimental

Retrorsine

Finely ground leaves and stems (6.3 kilos) of *Senecio retrorsus* were extracted in a Soxhlet extractor with purified methanol until no further extraction took place. Citric acid was added to the extract until it was strongly acid to litmus and the greater portion of the solvent was removed on a steam bath. Water was added to the residue until no further precipitation ensued and the resin was allowed to settle for several days. The supernatant liquid was then filtered with suction and the residue thoroughly washed with water. The filtrate was then stirred up with a small quantity of charcoal and the small amount of methanol still remaining was removed on a steam bath in a current of air. The cooled mixture was filtered through a layer of filtercel and the filtrate extracted with chloroform, until no further color was removed.

The combined chloroform extracts on clarification and evaporation yielded a brown viscous residue which rapidly deposited large hexagonal crystals. The incorporation of a little alcohol facilitated the removal of the mother liquor at the pump. The cautiously washed crystals were redissolved in chloroform, the solution evaporated to a small volume and treated with much ether. The clear supernatant solution was decanted from a small amount of gummy precipitate, and evaporated to a small volume. While still warm the substance began to separate in the colorless characteristic plates. The mixture was again recrystallized by solution in a small volume of hot chloroform and addition of ether. As thus obtained the substance begins to sinter at 175°, shrinks considerably at 182-186° and melts to a clear liquid at 190° C. Analysis: Calcd. for $C_{12}H_{16}O_7$; C, 52.94; H, 5.88%; mol. wt. 272. Found: C, 53.58, 53.80; H, 6.10, 6.18%; mol. wt. 279, 277 (Rast).

The aqueous solution from the above chloroform extract was again filtered through a layer of charcoal to remove a small amount of dark resin which had separated during the chloroform extraction. After the removal of the chloroform the mixture was made distinctly alkaline with potassium hydroxide and allowed to remain at room temperature for several days, during which time an alkaloid gradually separated in crystalline form. This was filtered off and the filtrate exhaustively extracted with chloroform. It had previously been observed that the base which crystallized directly was identical with that which

was removed by chloroform extraction, so the chloroform extract was added to the crystalline material and the mixture heated until solution was complete, filtered with the aid of charcoal, and evaporated to incipient crystallization. A small volume of methanol was added and crystallization allowed to proceed to completion at a low temperature. The base was filtered off and washed with cold methanol. The filtrate and washings were combined, acidified with citric acid, diluted with water and the remaining methanol boiled off. The deposited resin was filtered off through charcoal and some further impurities removed by extraction with chloroform. The alkaloid was removed by means of chloroform from the basified solution and crystallized as described above. The final mother liquor on concentration to a small volume deposited some more base which was combined with the first two crops. A repetition of the solution in acid and removal of impurities yielded a further small amount of the same base. The total yield of this crystalline product, which melted at about 212° C., darkening taking place at 208° C., was 81.8 gm. (1.3%).

The final mother liquors which yielded no further crystalline material, due no doubt to the accumulation of impurities, was treated in chloroform solution with methyl iodide. A rapid separation of a methiodide took place and recrystallization of the product yielded the characteristic substance shortly to be described. It therefore appears that no other base is present in appreciable quantity in *S retrorsus*.

Purification of the above base was effected in various ways, namely, recrystallization from acetone, from chloroform, from a large volume of methanol, and in no case separation into two or more substances could be effected. The crystals when slowly formed occur in short, stout tetragonal prisms one end of which is frequently developed obliquely. The melting point of the repeatedly recrystallized substance is indefinite at 214-215° C. with darkening and frothing, sintering taking place a few degrees lower. It gives no coloration with Ehrlich's reagent but an observation of undoubted structural significance is that the decomposed melt gives an immediate reddish-purple tint. The same is true of the overheated retronecine hydrochloride and undoubtedly indicates a latent pyrrol nucleus, which requires only dehydration to pass into the aromatic type. Analysis: Calcd. for $C_{18}H_{25}O_6N$; C, 61.54; H, 7.12; N, 3.99%. Found: C, 60.95; H, 7.14; N, 4.01%.

This analysis is the mean of three concordant combustions. It will be observed that the value for carbon is slightly low, and this has also been found to be the case with jacobine and the base from *S. vulgaris*. The derivatives and hydrolytic products however give more satisfactory figures.

Retrorsine Methiodide

When methyl iodide is added to a chloroform solution of retrorsine containing a small amount of alcohol a rapid separation of the methiodide in crystalline form takes place. The product is soluble with difficulty in hot alcohol and moderately soluble in hot water from which it crystallizes in brilliant stout prisms with blunt pyramidal terminations. It darkens at 256° C. and swells up and chars completely at 266° C. Analysis: Calcd. for $C_{19}H_{28}O_6IN$; C, 46.25; H, 5.68; N, 2.84; I, 25.76%. Found: C, 46.51; H, 5.69; N, 2.71, I, 26.05%.

Retronecine and Retronecic Monolactone

A solution of 2.5 gm. of retrorsine in 75 cc. of hot absolute alcohol was treated with 3 gm. of potassium hydroxide dissolved in 2 cc. of water. In the course of about 10 min. the potassium salt of retronecic acid began to separate in fine colorless needles. The mixture was allowed to remain overnight, then heated to boiling, cooled, and the separated salt filtered off and washed with cold absolute alcohol.

The filtrate was made just acid to Congo red with conc. hydrochloric acid, some potassium chloride filtered off and the alcohol evaporated from the filtrate. The dry residue was thoroughly washed with hot ethyl acetate which removed a small amount of retronecic acid. The slightly colored crystalline residue was freed of potassium chloride by several recrystallizations from absolute alcohol. In the final purification for analysis a concentrated alcoholic solution was treated with ethyl acetate until a considerable turbidity had developed. The mixture was rapidly filtered, the filtrate evaporated somewhat and cautiously treated with acetone. Retrorsine hydrochloride as thus obtained consists of transparent tetragonal plates, containing no water of crystallization. It melts sharply at 164° C. to a clear colorless liquid. Analysis: Calc. for $C_8H_{13}O_2N.HCl$: C, 50.14; H, 7.31; N, 7.31; Cl, 18.52%. Found: C, 50.26; H, 7.44; N, 7.00; Cl, 18.52%.

The retronecic monolactone is conveniently obtained from the above potassium salt by treatment with conc. hydrochloric acid until acid to Congo red, evaporation to dryness on the steam bath in a current of air, and extraction with hot ethyl acetate. The extract on treatment with charcoal and evaporation to a small volume yielded the crystalline substance on cooling. When slowly crystallized from ethyl acetate it was obtained in elongated needles generally terminated on one end with a pyramid and on the other with an oblique face. It is sparingly soluble in cold ethyl acetate and acetone, and almost insoluble in ether. No solvent is lost on heating. The pure substance melts at 186° C. and solidifies on cooling. The melting point of the resolidified melt is a few degrees lower. Analysis:— Calcd. for $C_{10}H_{14}O_5$; C, 56.08; H, 6.54%. Found: C, 55.90; H, 6.68%.

Benzoyl-retronecine Hydrochloride

A mixture of retronecine hydrochloride and powdered potassium carbonate was treated with an excess of benzoyl chloride in chloroform, and gently heated under reflux for 30 min. The chloroform solution was washed with water and the base extracted with dilute citric acid solution. The base was regenerated by the addition of alkali and extracted with chloroform. The extract was clarified over potassium carbonate and the solvent removed. Benzoyl retronecine as thus obtained consisted of a colorless viscous syrup readily soluble in ether. It failed to crystallize in contact with a number of solvents.

The hydrochloride in the amorphous state is readily soluble in acetone but when once obtained crystalline dissolves with difficulty. It was recrystallized by seeding a solution in acetone with a nucleus that had separated from another preparation. It consists of colorless needles, melting at 151° C.

Analysis:— Calcd. for $C_{15}H_{17}O_3N.HCl$; C, 60.92; H, 6.09; N, 4.74; Cl, 12.00%. Found: C, 60.56; H, 6.37; N, 4.85; Cl, 11.67%.

Benzoyl-retronecine Methochloride

The free base on treatment with methyl iodide in chloroform yielded an immediate precipitate of the methiodide which however was not obtained crystalline. It was readily soluble in acetone. The methiodide was converted into the methochloride by heating in aqueous solution with an excess of silver chloride. The filtrate was evaporated to a small volume. Crystallization occurred while the solution was still warm, and was hastened by the addition of acetone. The substance was recrystallized from alcohol-ether. As thus obtained it consists of stout, irregular plates, melting at 128° C. Analysis:— Calcd. for $C_{16}H_{20}O_3NCl$; C, 62.04; H, 6.47; N, 4.52; Cl, 11.46%. Found: C, 60.40; H, 6.72; N, 4.40; Cl, 11.24%.

Di-p-phenylphenacyl Ester of Retronecic Acid

The dried potassium salt of retronecic acid was accurately weighed and treated with exactly two moles of *p*-phenylphenacyl bromide in sufficient 90% alcohol to effect complete solution when hot. In the course of heating for three hours some of the ester had crystallized. Water was cautiously added until the incipient turbidity just disappeared on mixing. When crystallization was complete the solid was filtered off, washed with cold alcohol in which it is sparingly soluble and recrystallized twice from acetone. The latter operation was conveniently effected by evaporating the charcoaled solution to a small volume, adding alcohol and slowly evaporating most of the remaining acetone. Slow cooling yielded a mass of minute needles which melt sharply at 155° C. Analysis:— Calcd. for $C_{38}H_{35}O_6$; C, 73.55; H, 5.81%. Found: C, 73.65; H, 5.85%.

Jacobine

The procedure outlined for the isolation of retrorsine from *S. retrorsus*, when applied to *S. jacobaea* with negligible modifications yielded a chloroform extract which on evaporation to a syrup and treatment with alcohol crystallized in a short time. The yield of crystalline product of this grade was only 4.1 gm. from 9.9 kilos of dried material, and only 0.4 gm. was recoverable from the mother liquor.

Jacobine is readily recrystallizable by the addition of methanol to a concentrated chloroform solution. It is only sparingly soluble in alcohol and very sparingly in ether. When slowly crystallized it may be obtained in flat elongated plates with pyramidal terminations. The purest specimen thus far obtained melted at 223-224° C. with vigorous decomposition, some sintering and darkening taking place several degrees lower. Although the pure alkaloid gives no reaction with Ehrlich's reagent the decomposed melt gives an immediate coloration. Analysis:— Calcd. for $C_{18}H_{23}O_6N$; C, 64.86; H, 6.91; N, 4.20%. Found: C, 63.89; H, 7.24; N, 4.35%.

The basic aqueous solution from which the alkaloid had been extracted with chloroform was acidified with acetic acid and boiled to expel chloroform. The charcoaled solution was treated with an aqueous solution of phenylhydrazine

acetate and gently warmed until a dark resin began to separate in small quantity. Charcoal was added and the mixture rapidly filtered with suction. Gentle warming then caused the separation of copper-colored crystalline flakes. The solution was allowed to cool and after 24 hr. the solid was filtered off. (The filtrate on heating for a short time yielded glucosazone in quantity. It was purified by washing with acetone and recrystallizing from hot alcohol by the addition of an equal volume of hot water; m.p. 208° C.) The thoroughly washed phenylhydrazone was dried, dissolved in hot acetone, in which it is quite soluble, and crystallized out by the cautious addition of water. A repetition of the process using alcohol instead of acetone yielded delicate brilliant golden plates, melting sharply at 178° C. The yield of purified product was 70 gm. Admixture with an equal weight of protocatechuic aldehyde phenylhydrazone caused a depression of some 20° C. together with decomposition at 162° C. Analysis:— Calcd. for $C_{13}H_{12}O_2N_2$: C, 68.42; H, 5.26; N, 12.28%; mol. wt. 228. Found: C, 69.14; H, 5.18; N, 12.01; mol. wt. 242 (Rast).

Treatment with dilute or conc. hydrochloric acid with or without formaldehyde leads to extensive resinification. The color is almost certainly inherent in the pure substance, since recrystallization from hot water, in which it is only sparingly soluble, with the aid of charcoal causes no decrease in intensity. The phenylhydrazone is moderately soluble in ether and in chloroform.

Jacobine Methiodide

A solution of jacobine in chloroform quickly deposited the crystalline methiodide when a small amount of methyl iodide was added. The substance is sparingly soluble in alcohol and was recrystallized from water, in which it is moderately soluble even in the cold, by the addition of alcohol. The mixture was filtered off and the crystals were washed with alcohol and then with acetone; colorless flat plates, darkening at 238° and decomposing at 252° C. Analysis:— Calcd. for $C_{18}H_{28}O_8NI$: C, 46.65; H, 5.62; N, 3.02%. Calcd. for $C_{19}H_{28}O_8NI$; C, 48.00; H, 5.47; N, 2.95%. Found: C, 47.41; H, 5.54; N, 3.10%.

Hydrolysis of Jacobine

The procedure adopted in the case of retrorsine when applied to jacobine yielded no sparingly soluble potassium salt. The alcoholic solution was therefore acidified to Congo red with conc. hydrochloric acid and the precipitated potassium chloride filtered off. The filtrate was evaporated to dryness on the steam bath in a rapid current of air and the residue exhaustively extracted with hot ethyl acetate. The insoluble portion yielded retronecine when purified as already described. The hydrochloride, alone or admixed with an authentic specimen, melted sharply at 164° C. and further comparison failed to disclose any differences.

The ethyl acetate extract was evaporated to a syrup and treated with much ether. A small amount of precipitate was obtained which proved to be retronecine hydrochloride. The ether solution on evaporation yielded a syrupy residue which crystallized readily when cautiously treated with petroleum ether. The solid was filtered off, washed with a little ether, in

which it is only sparingly soluble, and recrystallized from much boiling ether.

Jaconecic acid thus obtained crystallizes in stellate aggregates of very fine needles, melting sharply at 178-179° C. Admixed with retronecic acid the mixture sinters at 152° C. and melts completely at 166° C. Analysis:— Calcd. for $C_{10}H_{16}O_6$: C, 51.72; H, 6.90%. Found: C, 52.30; H, 7.03%.

Examination of S. aureus for Alkaloids

Dried *S. aureus* (2.25 kilos) obtained from Eimer and Amend of New York was ground to a fine powder and thoroughly extracted with methanol in a Soxhlet extractor. The extract was treated as in the case of *S. retrorsus*. The chloroform extract after removal of non-basic material on evaporation yielded less than 0.5 gm. of a pale yellow resin which could not be obtained crystalline. That a small amount of an alkaloid is present was indicated by the fact that treatment with methyl iodide yielded a trace of a water soluble substance which gave several isolated crystals when the solvent was removed and the residue left in contact with a little methanol. The quantity obtained, however, was insufficient for isolation in a state of purity.

It is therefore not justifiable to state with certainty that an alkaloid is present.

References

1. CUSHNY, A. R. Proc. Roy. Soc. Lond. B. 84: 188-190. 1912.
2. DRAKE, N. L. and BRONITSKY, J. J. Am. Chem. Soc. 52: 3715-3720. 1930.
3. GRANDVAL, A. and LAJOUX, H. Compt. rend. 120: 1120-1123. 1895.
4. SHIMOYAMA, Y. Apoth. Ztg. 7: 453-454. 1892.
5. WATT, H. E. J. Chem. Soc. 95: 466-477. 1909.
6. WEGSCHEIDER, R. Monatsh. 17: 245-252. 1896.

SPONTANEOUS SELF-FERTILIZATION IN RELATION TO SEED PRODUCTION IN SWEET CLOVER (MELILOTUS)[1]

By L. E. Kirk[2] and T. M. Stevenson[3]

Abstract

The chief determining factor in spontaneous self-fertilization in white sweet clover (*Melilotus alba*) appears to be the distribution of pollen within the unopened flowers. This in turn depends on (1) length of stamens, (2) length of style, (3) amount of pollen, and (4) size of cavity within the upper part of the keel. When the flower structure and quantity of pollen is such as to insure the deposition of pollen grains on the stigma before the blossom is likely to be disturbed by insects, the plant will be normally self-fertilized. In the yellow flowered species of sweet clover (*Melilotus officinalis*) spontaneous self-fertilization is effectively prevented, except in certain varieties, by a characteristic of the stigma which makes it unreceptive. Scarification of the stigmatic surface appears to be necessary before fertilization can take place. Variation occurs also, as in *M. alba*, in the length of stamens and style, size of keel cavity, and the amount of pollen.

There is a high correlation in *M. alba* between the percentage of flowers which are naturally self-pollinated and the percentage of flowers which produce pods when the plants are caged to exclude insects. Selection of plants which are normally self-fertilized can be made by examining the early flowers, thus obviating the necessity of bagging or caging plants which do not possess this character.

A strain of *M. alba* which is normally self-fertilized was found to produce almost twice as much seed as another strain which is normally cross-pollinated under comparable conditions in the field.

In a recent article, the writers (1) described certain characteristics of flower structure and behavior in *Melilotus alba* and *Melilotus officinalis* and their relation to the degree of self-fertilization in these species. The results of experiments which were submitted were based on a laboratory study of plants grown in the greenhouse. During the summer season of 1931 an extensive survey was made of a large amount of plant material in the sweet clover breeding nursery for the purpose of finding whether the facts previously ascertained could be verified under field conditions and also to what extent seed production might be affected in actual practice. The results of experiments with plants in the field seem to the writers of sufficient interest to warrant another report of a supplementary nature which may best be read in conjunction with the paper cited above.

These experiments included several varieties of both *M. alba* and *M. officinalis*, some of which were not examined previously, as well as a number of inbred lines of *M. alba*. The same methods were followed as described in the first article. Only flowers with petals unopened or only partially expanded, and which had not been visited by bees, were used. Those which have been visited by honey bees are easily detected because the edges of the keel, when once forced apart, remain partially opened, whereas they are closely pressed together in flowers which have not been disturbed. The flowers were treated

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with alcohol and cedar clearing oil, as previously described, to make them transparent and excellent results were always secured with both the white and yellow flowered species providing examination was made at the proper time. If examination is delayed for some time after the flowers become transparent they take on a decidedly abnormal appearance.

Flower Structure and Self-fertilization

Seven factors which appeared to influence self-fertilization were described in the previous article. These were (1) length of stamens, (2) amount of pollen, (3) stage of flower development when pollen is liberated from the anther sacs, (4) receptivity of the stigma, (5) distribution of free pollen within the flower, (6) size of cavity in the upper part of the keel, (7) condition of the pollen. Recent observations justify certain modifications in this list and enable us to obtain a better idea of the different factors as to their probable relative importance.

The relative position of anthers and stigma was thought to depend almost entirely on the length of the filaments. A more extensive examination of varieties and inbred lines has revealed a marked variation also in "length of style". Certain of the inbred lines showed very pronounced differences in this character. Some idea of the comparative lengths of styles may be obtained by observing in Fig. 1 the position of the stigma in relation to the highest point of the keel in the two flowers. The difference is not due to length of keel because it was found by working with a very large number of flowers from different plants in the two selfed lines represented, that the one with the shorter style also had the shorter keel. This would tend to minimize rather than accentuate the apparent difference.

The fact seems to be that both filaments and style are subject to variation in length and it is not always an easy matter to decide which one determines the position of the anthers in relation to the stigma. In many cases it is probable that the length of both are involved at the same time.

With respect to the stage of flower development when pollen is liberated from the anthers it may be said that even less variation was found in the field material than in the greenhouse material. Not a single case was found in which the pollen was not normally liberated from the anthers before the flowers were fully opened. In fact, almost all of the flowers examined contained some anthers which had dehisced before the petals were even partially expanded.

With respect to the germination of pollen grains within the unopened flower which was reported in the earlier paper, it must be said that this character did not show up under field conditions. In the greenhouse studies, germination of pollen within the flower was found to occur to a marked extent in the variety "Redfield Yellow" and also in one plant of *M. alba*. It was not observed in a single one of the numerous plants which were grown in the field including plants of the Redfield Yellow variety. It is difficult, but not illogical, to consider the divergent results as being due to different environmental conditions because, in the same greenhouse, only two out of several varieties and strains exhibited this character. There is still the possibility that inherent differences

in plant material were responsible in view of the fact that observations in the greenhouse were made on a relatively small number of plants, and these special types may not have been encountered in the field.

The chief determining factor in spontaneous self-fertilization in *M. alba* appears to be the distribution of pollen within the unopened flower, since there is a high correlation between the number of flowers which have pollen on the stigma and the degree of actual seed setting. This in turn is determined by four major factors as follows: 1. Length of stamens. 2. Length of style. 3. Amount of pollen. 4. Size of keel cavity. To these a fifth may be added, namely, "receptivity of the stigma", which however appears to be important only in certain types of *M. officinalis*. Table I gives the results of observations in several selfed lines of *M. alba* and shows the combinations of factors which resulted in various percentages of self-pollinated flowers together with the actual percentage of seed setting when the plants were protected from the visitation of insects.

TABLE I

DIFFERENT COMBINATIONS OF FACTORS IN SELFED LINES OF *M. alba* AS THEY AFFECT POLLINATION AND SEED SETTING OF PROTECTED PLANTS

Selfed line	Factors affecting distribution of pollen				Percentage of flowers having pollen on the stigma	Percentage of flowers having produced pods
	Length of stamens	Length of style	Amount of pollen	Size of cavity in upper part of keel		
1	Long	Intermediate	Abundant	Large	100	95.50
2	Intermediate	Short	Scarce	Intermediate	86	80.00
3	Short	Intermediate	Abundant	Intermediate	80	70.60
4	Short	Long	Abundant	Small	55	55.80
5	Intermediate	Long	Intermediate	Small	65	58.80
6	Short	Intermediate	Intermediate	Large	20	18.70
7	Long	Long	Scarce	Intermediate	20	18.00
8	Intermediate	Intermediate	Intermediate	Intermediate	14	10.00
9	Short	Intermediate	Scarce	Small	14	12.00
10	Intermediate	Long	Scarce	Intermediate	3	3.00
11	Intermediate	Long	Very scarce	Intermediate	0	0.00

It is evident from Table I that there was a high correlation between the number of flowers which received pollen on the stigma and the number which produced pods. Line 1 may be considered as 100% self-fertilized, since the examination of more than 100 flowers on each of 34 plants of this strain did not reveal a single blossom that had not been pollinated before being disturbed by insects. Actual counts showed that 95.5% of the flowers produced pods under closely woven cotton cages in the field nursery.

In the process of breeding by selection within self-fertilized lines it may be an important matter to determine before caging, which plants are naturally self-pollinated. This can be done quite easily by examining the first blossoms which appear and observing the relative number of undisturbed flowers which have pollen grains deposited on the stigma. If it is the object to secure lines only which are spontaneously self-fertilized, this method of procedure will obviate the necessity of working with large numbers of unsatisfactory plants.

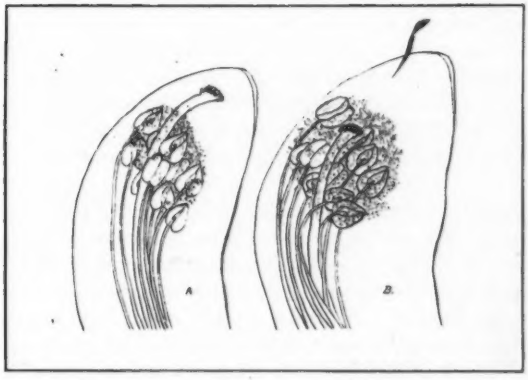

FIG. 1. *Typical flowers from two selfed lines of M. alba showing variation in length of style.*

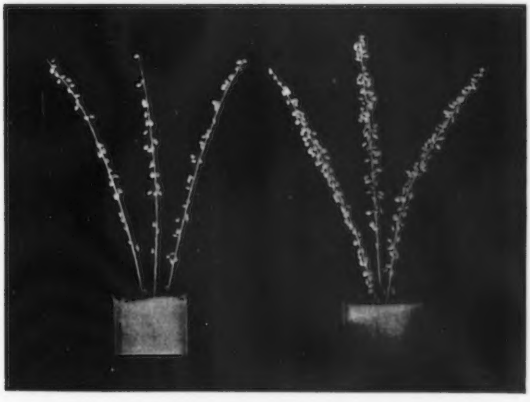

FIG. 2. *Typical seed setting in two selfed lines of M. alba under field conditions showing effect of spontaneous self-fertilization. Left, a normally cross-pollinated strain. Right, a normally self-pollinated strain.*

Self-fertilization in Relation to Seed Setting in M. alba

The value of spontaneously self-fertilized strains of sweet clover is very obvious from the standpoint of maintaining the purity of varieties which possess valuable qualities. This would be a consideration in the case of strains which were bred for resistance to disease. In this connection the question arises also whether strains which are naturally self-pollinated may be expected to produce more seed than others which are naturally insect-pollinated when both are grown under the same conditions and exposed equally to the visitation of insects.

Inbred lines of *M. alba* in the field nursery, which were normally self-fertilized in varying degrees, provided excellent material for estimating the effect of natural self-pollination on the amount of seed produced under ordinary field conditions. For this purpose, five selfed lines were chosen differing widely in the percentage of undisturbed flowers which had been self-pollinated. About 25 plants were examined in each line, and counts made of both flowers and pods on 10 normal racemes from each plant. The number of flowers on each raceme can be determined from the points of attachment on the axis. These are clearly visible with a low power lens. The selfed lines were grown close to one another in the nursery and all were exposed equally to the visitation of honey bees, which were present in large numbers, a large apiary being located nearby.

In the case of plants in the same selfed lines, from which insects were excluded by means of large cotton cages, the determinations on seed setting were made in the same manner as for open-pollinated plants.

Table II gives the percentage of normally self-pollinated flowers in each strain and the percentage of flowers which produced pods when the plants were protected from, and exposed to, the visitation of insects.

TABLE II

RELATIVE SEED PRODUCTION IN SELFED LINES OF *M. alba* WHICH DIFFERED IN THE PERCENTAGE OF FLOWERS THAT PRODUCED SEED NORMALLY BY SELF-FERTILIZATION

Strain number	Percentage of flowers that had pollen on the stigma	Percentage of flowers that produced pods	
		Protected plants (self-pollinated)	Unprotected plants (open-pollinated)
1	100	95.5	99.1
3	80	70.6	73.9
4	55	55.8	68.1
5	65	58.8	74.6
11	0	0	54.1

In Table II, strain 1 represents a selfed line which is completely self-pollinated under all conditions and strain 11, a selfed line which will not normally produce seed unless the flowers have been artificially manipulated or pollinated by insects. It is interesting to observe that strain 1 produced almost twice as many pods per hundred flowers as did strain 11 under conditions favorable for open pollination. Furthermore the amount of seed setting appears to have

been considerably below the optimum in the other strains which were only partially self-fertilized.

The evidence submitted seems to show that strains which are spontaneously self-fertilized may be expected to produce considerably more seed than those which are partially or wholly dependent on insect pollination. This is what one would expect since it was evident from an examination of the older flowers that the bees did not work on every blossom, nor was there pollen on the stigma of every flower which had been visited.

Fig. 2 shows the seed setting on typical racemes at maturity from strain 11 (left) which is normally cross-pollinated, and strain 1 (right) which is normally self-pollinated.

Acknowledgment

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Reference

1. KIRK, L. E. and STEVENSON, T. M. Factors which influence spontaneous self-fertilization in sweet clover (Melilotus). Can. J. Research, 5: 313-326. 1931.

THE TYPES OF OSMOPHILIC YEASTS FOUND IN NORMAL HONEY AND THEIR RELATION TO FERMENTATION[1]

By A. G. Lochhead[2] and Leone Farrell[3]

Abstract

A study of the predominant sugar-tolerant yeasts infecting 163 samples of normal Canadian honey led to the recognition of eight different species, comprising the genera *Zygosaccharomyces*, *Schizosaccharomyces* and *Torula*. The frequency of their occurrence varied greatly, one type, *Z. richteri*, being by far the most commonly encountered. The yeast predominating originally is not necessarily the most abundant type after fermentation. Analysis of samples fermenting within 14 months showed species of *Zygosaccharomyces* only to be most abundant, while *Z. richteri*, in addition to being the predominant type infecting a large majority of samples, was able, even in certain cases where it was originally outnumbered, to develop and apparently assume the leading role in fermentation.

Introduction

In a previous investigation reported from this laboratory (8), a study was made of the infection of normal honey by sugar-tolerant yeasts. An examination of 191 samples, representing all parts of Canada, showed the presence of yeasts in all cases though the amount of infection, as indicated by yeast counts, varied widely. It was found, moreover, that the tendency to ferment increased with increasing yeast infection, which latter was believed to be a factor directly affecting fermentation.

To note whether the types of osmophilic yeasts, as distinct from the numbers present, are of importance in causing fermentation the present study was undertaken. The yeast types occurring most abundantly in the original samples were first determined. The honeys were then placed in storage and examined for fermentation. From those which fermented within a 14-month storage period the most abundant yeast types were again isolated and studied.

Experimental

In the study referred to above, yeast counts were made by the dilution method, the medium employed consisting of two parts by weight of honey diluted by the addition of one part of a nutrient solution containing, per litre; peptone, 1.0 gm.; K_2HPO_4, 1.0 gm.; $MgSO_4$, 0.5 gm.; ammonium tartrate, 0.5 gm.; NaCl, 0.1 gm.; and $CaCl_2$, 0.1 gm. For the present study, the tubes which showed fermentation with the greatest dilutions of honey were used and from them yeasts were isolated by plating on 60% honey agar. Plates were incubated at 28° C. and isolations made from all colonies which exhibited any macroscopic differences. The cultures isolated were purified by replating three times on the same medium preparatory to the detailed morphological

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and physiological examination. In this way 201 cultures were obtained from 163 different samples of normal honey.

For comparison and identification of the cultures isolated, transfers were made on 50% honey agar slants, on 15% honey agar flasks for giant colony formation, and into dextrose, saccharose and maltose broth prepared by adding the sugar in 10% concentration to a basic solution of 0.5% yeast extract broth. Microscopic and macroscopic examination of the growth on solid media with a comparison of the fermentative characteristics permitted an elimination of many cultures. After this preliminary survey all remaining cultures were studied in greater detail according to the following plan:

(a) Microscopic observation on solid and liquid media.
(b) Growth on honey agar slants, containing 15% and 70% honey respectively.
(c) Growth in honey broth of 15% and 70% honey.
(d) Giant colony formation on 15% honey agar.
(e) Growth on carrot, potato, in milk and in gelatine media containing 15% and 70% honey respectively.
(f) Fermentation tests with the following: arabinose, xylose, dextrose, levulose, mannose, galactose, saccharose, maltose, lactose, raffinose, dextrin and mannite.
(g) Comparison of growth at 37° C. on honey agar of high and low concentration (70% and 15%).

As a result of these tests the cultures were finally reduced to eight types, sufficiently distinct as to morphology and cultural characteristics as to warrant being considered different species. In addition, two types, closely related to two of the species, were classed as subspecies. Of the eight types six were classified as *Zygosaccharomyces*, one as *Schizosaccharomyces* and one as *Torula*.

Duplicate samples of the honeys from which the above isolations were made were held in storage at room temperatures and observed for fermentation. Samples which were found to have fermented within a 14-month period were analyzed for total yeast count by the dilution method previously described (8). To determine the yeast types occurring most abundantly, isolations were made as before from the tubes showing fermentation with the highest dilution of honey. In all, 30 samples of fermented honey were examined and the cultures isolated were subjected to the same process of comparison as previously described for those from normal honey. In this manner, four species, with two related subspecies of sugar-tolerant yeasts, all of the genus *Zygosaccharomyces*, were distinguished.

The frequency with which the various types of sugar-tolerant yeasts were found as the predominating organism in normal honeys varied widely. As may be seen from Table I, one type, culture 20, occurred much more frequently than any of the others, some being isolated in but one instance. It will be noted, furthermore, that in the case of but three yeast types, representing a small fraction of the samples, did the honeys in question all remain unfermented. In all cases of fermentation the originally predominant organisms were found to be *Zygosaccharomyces*. The belief that members of this genus are primarily

concerned with fermentation is supported by the results of the examination of the fermented samples. In every instance the predominant yeast type proved to be of the genus *Zygosaccharomyces*.

From Table I it will be observed that in many cases the yeast type predominating originally did not prove to be that most abundant in the honey after fermentation. This leads to the belief that the organism with which a sample of honey may be most heavily infected is not necessarily the causal agent of fermentation. Thus culture 78, which was found as the predominant type in 23 samples, of which 7 fermented, was not isolated in any of these cases after fermentation. On the other hand, yeasts of the culture 20 group,

TABLE I

INCIDENCE OF OSMOPHILIC YEASTS IN NORMAL HONEY AND IN FERMENTED SAMPLES

Culture No.	Classification	Times predominant in normal honey	Samples fermenting within 14 months	% Fermented	Types predominating after fermentation								
					Types in original samples								Other types
					20	78	138	16X	155Y	139	58	11Y	
20	*Zygosaccharomyces*	120	22	18.3	20	0	1	0	0	0	0	0	1
78	*Zygosaccharomyces*	23	7	30.4	6	0	0	0	0	0	0	0	1
138	*Zygosaccharomyces*	16	4	25.0	0	0	2	0	0	0	0	0	2
16X	*Torula*	9	0	0.0	–	–	–	–	–	–	–	–	–
155Y	*Zygosaccharomyces*	8	2	25.0	1	1	0	0	0	0	0	0	0
139	*Zygosaccharomyces*	7	2	28.6	2	0	0	0	0	0	0	0	0
58	*Zygosaccharomyces*	1	0	0.0	–	–	–	–	–	–	–	–	–
11Y	*Schisosaccharomyces*	1	0	0.0	–	–	–	–	–	–	–	–	–

found as the predominant type in 120 samples of which 22 later fermented, was found after fermentation, not only in 20 of these cases, but also in 9 samples in which the originally predominant yeasts were of other types. Culture 20 and related types are considered to be *Zygosaccharomyces richteri*, first isolated from fermented honey (7), but also found as the most common yeast infecting hive nectar (7). It has also been isolated from the nectar of various flowers (7) and from apiary soil (6). Altogether it appears to be the most ubiquitous sugar-tolerant yeast concerned with honey. Not only is it the predominant yeast infecting a large majority of samples, but even in cases where it is outnumbered by other types, it is apparently able to develop and assume the leading role in honey fermentation.

Yeasts Isolated from Normal Honey

Of the eight species isolated from normal honey, five appeared to be identical with yeasts already reported from this laboratory (6, 7), and consequently a detailed description of their morphological and cultural characteristics is not repeated here. Their classification, however, may be briefly indicated.

CULTURE 20 (*Zygosaccharomyces richteri*) (see Fig. 1)

This yeast, which ferments dextrose, levulose, and mannose is considered identical with culture M1, isolated from fermented honey (7), and culture S3B3 found in apiary soil (6) and previously described. Some strains show a

slight tendency to ferment saccharose. On 15% honey agar the giant colony is normally smooth at first with concentric and radial markings, the surface becoming later covered with a dotted growth. The form of growth, however, is less characteristic than in the case of most of the other species found.

CULTURE 78 (*Zygosaccharomyces nussbaumeri*) (see Fig. 2)

This yeast, which shows a characteristically raised and folded growth on such media as 15% honey agar and carrot, ferments dextrose, levulose, mannose, saccharose and maltose. It has been previously described as culture J7 from fermented honey (7) and culture S3B6 from apiary soil (6).

CULTURE 16X (*Torula sp.*) (see Fig. 3)

Forming a very characteristic giant colony on 15% honey agar, this yeast is readily distinguished. Fermenting dextrose, levulose, mannose, saccharose and raffinose, it is identical with culture S3C3, isolated from apiary soil and previously described (6). From one sample a culture (11X), considered a subspecies of 16X, was isolated. While agreeing with the latter in other respects, it was unable to cause fermentation of saccharose and raffinose. Otherwise the description will suffice.

CULTURE 155Y (*Zygosaccharomyces sp.*) (see Fig. 4)

The growth of this yeast on such media as 15% honey agar, carrot, potato is usually slow and scanty. On 15% honey agar colonies are characteristically small and raised. Dextrose, levulose and mannose are fermented. With this yeast fermentation is much more vigorous in 70% than in 15% honey broth. This type is regarded as the same as N4, from floral nectar (7), and culture S3B2, isolated from apiary soil and previously described (6).

CULTURE 139 (*Zygosaccharomyces sp.*) (see Fig. 5)

This yeast produces a characteristic growth on 15% honey agar. The surface of the colony, at first smooth, becomes later heaped up into coarse folds particularly in the central portion, facilitating recognition. Dextrose, levulose, mannose, saccharose and maltose are fermented. This yeast is identical with culture S3B11, isolated frequently from apiary soil (6).

In addition to the five species indicated above, three species were isolated from normal honey, the descriptions of which may be given in greater detail.

CULTURE 138 (*Zygosaccharomyces sp.*) (see Fig. 6)

In young cultures on 15% honey agar, cells are ellipsoidal to round, occurring singly, in pairs and in small groups and chains of adherent cells. The cells vary in length from 3 to 7 μ and in width from 2 to 4.5 μ, the average size being 4.5 by 3 μ. In old cultures on 70% honey agar there is some tendency to form hyphae of elongated cells. Reproduces asexually by budding. Spore formation, resulting from isogamic copulation may be observed on such media as honey agar, honey gelatine and honey broth. Ascospores were observed regularly to be two in number, of a diameter of approximately 3 0 μ.

Honey agar 15%.—Abundant filiform growth, light cream colored, with surface smooth at first but usually somewhat folded in older cultures; edge finely lobate; lustre dull; soft cheesy consistency.

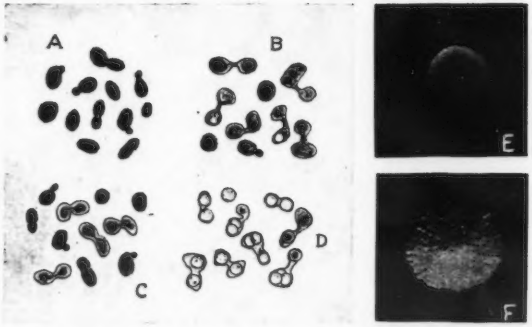

FIG. 1. CULTURE 20. *A. Young culture, honey agar 15%, three days, showing budding. B. Ten-week culture, honey agar 70%, showing copulation and ascospore formation. C. Carrot culture, eight days, showing copulation. D. Culture on honey gelatine, nine weeks, showing ascospores. E. Giant colony, honey agar 15%, 34 days. F. Giant colony, honey agar 15%, 164 days.*

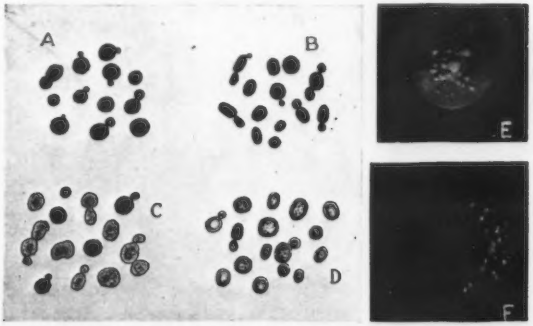

FIG. 3. CULTURE 16X. *A. Young culture, honey agar 15%, three days, mostly round cells, showing budding. B. Potato culture, seven days, showing ellipsoidal cells. C. Nine-week culture, honey agar 70%. D. Culture on carrot, nine weeks, showing thick-walled cells. E. Giant colony, honey agar 15%, 34 days. F. Giant colony, honey agar 15%, 110 days.*

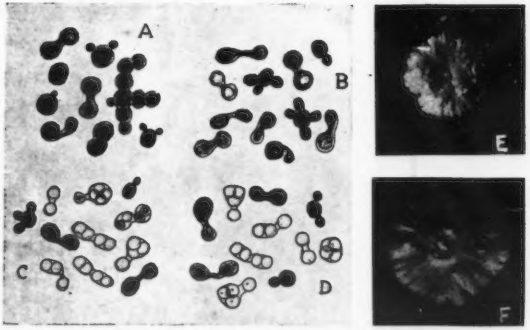

FIG. 2. CULTURE 78. *A. Young culture, honey agar 15%, three days. B. Eight-day culture on carrot, showing copulation and ascospore formation. C. Ten-day culture, honey agar 15%, showing ascospores. D. Culture on honey agar 70%, 10 weeks, showing ascospore formation. E. Giant colony, honey agar 15%, 34 days. F. Giant colony, honey agar 15%, 110 days.*

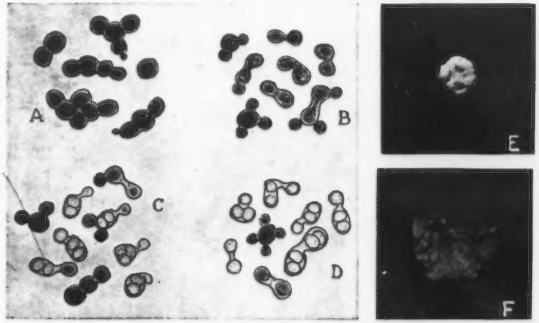

FIG. 4. CULTURE 155Y. *A. Young culture, honey agar 15%, three days, showing budding. B. Eight-day culture, honey agar 15%, showing copulation. C. Carrot culture, nine weeks, showing ascospores. D. Nine-week culture, honey agar 70%, showing ascospores. E. Giant colony, honey agar 15%, 34 days. F. Giant colony, honey agar 15%, 146 days.*

PLATE I

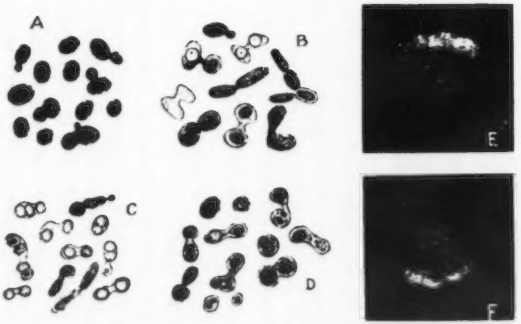

FIG. 5. CULTURE 139. *A. Young culture, honey agar 15%, three days, vegetative cells. B. Four-week carrot culture, showing copulation and ascospore formation. C. Nine-week carrot culture, showing ascospores, also elongated cells. D. Culture on honey gelatine 15%, showing ascospore formation by copulation, also apparently by parthenogenesis. E. Giant colony, honey agar 15%, 34 days. F. Giant colony, honey agar 15%, 50 days.*

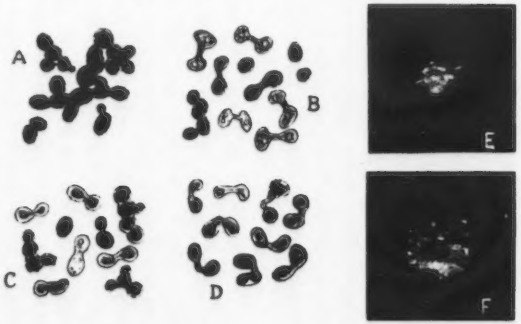

FIG. 7. CULTURE 58. *A. Young culture, honey agar 15%, four days, vegetative cells. B. Ten-week culture, honey agar 70%, showing copulation C. Carrot culture, 15 days, showing copulation, also thick-walled vegetative cells. D. Four-week culture on potato, showing copulation and ascospore formation. E. Giant colony, honey agar 15%, 34 days. F. Giant colony, honey agar 15%, 50 days.*

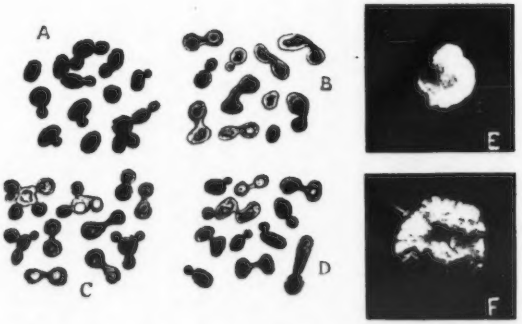

FIG. 6. CULTURE 138. *A. Young culture, honey agar 15%, three days, vegetative cells. B. Culture on honey gelatine 15%, eleven weeks, showing copulation. C. Surface ring growth, honey broth 15%, eleven weeks, showing copulation and ascospore formation. D. Eleven-week culture, honey agar 70%, showing ascospore formation, also elongated cells. E. Giant colony, honey agar 15%, 22 days. F. Giant colony, honey agar 15%, 74 days.*

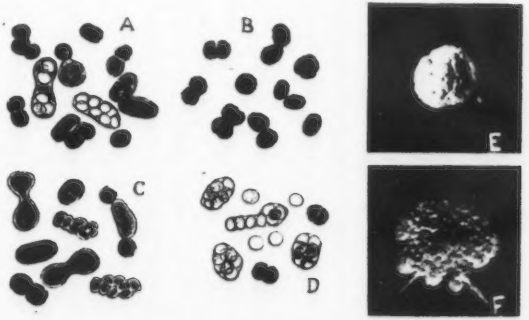

FIG. 8. CULTURE 11Y. *A. Young culture, honey agar 15%, showing vegetative cells, also ascospores. B. Surface ring growth, honey broth 70%, showing multiplication by fission. C. Seven-day culture, honey agar 70%, showing copulation. D. Eight-day culture, honey agar 15%, showing ascospores. E. Giant colony, honey agar 15%, 34 days. F. Giant colony, honey agar 15%, 50 days.*

Honey agar 70%.—Moderate filiform growth, light brown in color, smooth except where surface may be broken by gas; somewhat glistening; consistency slightly viscous.

Honey broth 15%.—Active alcoholic fermentation; surface ring growth; abundant flocculent sediment with liquid finally becoming clear.

Honey broth 70%.—Active fermentation; brownish surface ring growth.

Giant colony, honey agar 15%.—General form of colony round, with margin very irregular and lobate; raised with irregular broken surface with concentric and radial markings; cream to flesh colored, tending to darken with age; dull lustre and soft cheesy consistency.

Carrot.—Growth abundant, somewhat spreading and raised, cream colored; surface contoured and smooth, at first glistening, though less so in older cultures; soft, butyrous consistency; medium unchanged.

Potato.—Moderate cream colored growth, somewhat raised, with contoured surface; slightly glistening; butyrous to soft cheesy consistency; medium unchanged.

Milk.—After 10 weeks, no visible change.

Gelatine.—No liquefaction observed after 10 weeks in honey gelatine 15% and 70%.

Growth at 37° C.—Growth on 70% honey agar, none on 15%.

Fermentations.—Dextrose, levulose, mannose and maltose are fermented with acid and gas formation. (A closely related type, culture 9, while similar in all other respects, did not ferment maltose and was classed as a subspecies.) No fermentation was observed with arabinose, xylose, galactose, saccharose, lactose, raffinose, dextrin and mannite.

The characteristics of this culture appear to coincide well with those of the yeasts of Group I of Fabian and Quinet (2), and is accordingly classed with this group. These workers regarded their type as *Zygosaccharomyces japonicus* Saito. With our culture as with theirs, however, film formation, a characteristic of *Z. japonicus*, was not observed. We are inclined to regard our yeast as related to *Zygosaccharomyces priorianus* Klöcker, isolated from the bodies of bees (4) and showing the same fermentative properties. Osmophilic yeasts, closely related to *Z. priorianus* and capable of causing fermentation in the concentrated wine must of "Trockenbeerenauslesen" have been isolated recently by Kroemer and Krumbholz (5).

CULTURE 58 (*Zygosaccharomyces sp.*) (see Fig. 7)

Young cultures on honey agar show mostly ellipsoidal cells though occasionally cylindrical types may be noted and a few round forms. Cells occur generally in groups formed by budding, with single cells and pairs being less frequently seen. There is considerable variation in size, cells ranging from 2.5 to 8 μ long by 2 to 5 μ in width. The majority of the cells are 3.5 to 5 μ long by 3 to 4 μ wide. In 70% honey broth, the cells are somewhat smaller. Old cultures on 70% honey agar show rather thick-walled cells, round to elongated with the occasional appearance of rudimentary mycelium. Reproduces asexually by budding. Zygospores are formed as a result of isogamic

copulation on such media as potato and 70% honey agar, ascospores appearing most frequently to the number of two, though one to three in the ascus were sometimes noted.

Honey agar 15%.—Moderate filiform growth, cream to fawn colored, with irregular, lobate edge; slightly raised, with surface glistening at first, but later becoming dull as it assumes a granular appearance; consistency firmly butyrous.

Honey agar 70%.—Moderate light brown, filiform growth, slightly raised, with smooth surface, except where broken by gas; margin finely wrinkled; slightly viscous consistency.

Honey broth 15%.—Active fermentation; light brown surface ring growth; abundant, coarsely flocculent sediment with liquid clearing.

Honey broth 70%.—Active fermentation; brownish surface ring growth.

Giant colony, honey agar 15%.—Colony rather small, irregular in shape with lobate edge; raised with irregular surface which assumes a characteristic, finely granular appearance; dark cream colored with dull lustre; consistency butyrous to cheesy.

Carrot.—Abundant, cream colored growth, slightly raised, with smooth, contoured surface; at first glistening; soft butyrous consistency; medium unchanged.

Potato.—Growth less abundant than on carrot; raised with dull surface; cheesy consistency; medium unchanged.

Milk.—No change observed after 10 weeks.

Gelatine.—Liquefaction of honey gelatine 15%; none observed in 70% after 10 weeks.

Growth at 37° C.—Good growth on honey agar 70%, only slight growth on 15%.

Fermentations.—Dextrose, levulose, mannose and saccharose are fermented. No fermentation observed with arabinose, xylose, galactose, maltose, lactose, raffinose, dextrin and mannite.

This yeast has the same fermentative properties as Group II of Fabian and Quinet (2) and culture E6, previously isolated from fermented honey in this laboratory (7). The above types were regarded as related to *Zygosaccharomyces barkeri* Saccardo-Sydow. Culture 58, however, exhibits both cultural and physiological differences from these types, and is also distinguished from *Z. nadsonii* by its isogamic copulation, It has, therefore, not been identified with other described species.

Culture 11Y (*Schizosaccharomyces octosporus*) (see Fig. 8)

From one sample of honey a yeast belonging to the comparatively rare genus *Schizosaccharomyces* was isolated. Members of this genus appear to be mainly tropical yeasts, and hence it was a matter of considerable interest to find a representative in a sample of Canadian honey. The honey in question was produced in the southern interior of British Columbia, in a warm dry district requiring extensive irrigation. It had the lowest moisture content of the samples examined, 15.9%.

In young cultures on honey agar 15%, round and elongated cells are seen,

about 5μ in diameter. Multiplication occurs by transverse division, a partition wall appearing in the middle of the cell. The two daughter cells become round in shape, and may finally separate or remain attached, and in their turn divide again into two cells by the same process of fission. Thus groups of 4 or more cells may be noted, giving an appearance similar to *Sarcina*. Ascospore formation occurs very readily on the media employed. It may occur as the result of a fusion of cells or apparently in elongated cells without copulation. The asci are comparatively large, 5 to 8 μ wide and 12 to 20 μ in length. The spores were found regularly to the number of 8 in the ascus, oval to round and measuring usually 4 to 5 μ long by 3.5 μ wide. Free spores appeared to be round.

Honey agar 15%.—Moderate filiform cream colored growth, soon becoming covered with globular surface outgrowths, light brown in color, and giving a verrucose appearance; lustre dull; consistency rather dry and cheesy.

Honey agar 70%.—Moderate filiform growth, light brown in color; slightly raised with surface becoming irregular, showing numerous ridges and cracks; dull, with butyrous consistency.

Honey broth 15%.—Alcoholic fermentation with gas; slight light brown ring growth round side of tube; abundant, finely divided sediment with liquid becoming clear.

Honey broth 70%.—Active alcoholic fermentation; brownish surface ring growth.

Giant colony, honey agar 15%.—Cream colored convex colony with irregular margin and smooth, rather dull surface on which secondary outgrowths occur, particularly in central portion; these globular outgrowths, darker in shade, finally cover the surface, giving the colony a characteristic verrucose appearance.

Carrot.—Growth slow and scanty, heaped up, giving a beaded appearance; dark cream colored and dull, with soft, cheesy consistency.

Potato.—Growth scanty, raised and beaded along line of inoculation; fawn colored at first, soon becoming chalky; dull, with cheesy consistency.

Milk.—After 10 weeks reaction slightly alkaline, otherwise no change.

Gelatine.—Liquefaction in honey gelatine 15%; none in 70% after 10 weeks.

Growth at 37° C.—None observed on honey agar 15% or 70%.

Fermentations.—Dextrose, levulose and maltose are fermented. No fermentation observed with arabinose, xylose, mannose, galactose, saccharose, lactose, raffinose, dextrin and mannite.

This yeast appears to correspond with *Schizosaccharomyces octosporus* isolated by Beijerinck from dried currants (1), and conforms well with the morphological and fermentative properties of this species as described by him. According to Guilliermond (3) *Sch. octosporus* is able to ferment other sugars than those indicated. Beijerinck, however, in testing a series of carbohydrates, found dextrose, levulose and maltose capable of being assimilated, with very sparse growth with mannite and dextrin. The author indicates good fermentation only in the case of dextrose, levulose and maltose.

Yeasts Isolated from Fermented Samples

Of the four types isolated from the samples after fermentation, three corresponded with yeasts from the original samples (see Table I). The fourth type, while agreeing in most characteristics with culture 20, fermented saccharose and maltose, and a comparison with yeasts previously described inclines us to classify it with Group IV of Fabian and Quinet (2).

References

1. BEIJERINCK, M. W. Centr. für Bakt. 16: 49-58. 1894.
2. FABIAN, F. W. and QUINET, R. I. Mich. Agr. Exp. Sta. Tech. Bull. 92. 1928.
3. GUILLIERMOND, A. The Yeasts (transl. by F. W. Tanner), Wiley. New York. 1920.
4. KLÖCKER, A. Handbuch der technischen Mykologie (F. Lafar). 2. Aufl. Jena. 4: 141-191. 1907.
5. KROEMER, K. und KRUMBHOLZ, G. Arch. für Mikrobiologie. 2: 352-410. 1931.
6. LOCHHEAD, A. G. and FARRELL, LEONE. Can. J. Research, 3: 51-64. 1930.
7. LOCHHEAD, A. G. and HERON, D. A. Dom. of Can. Dept. Agr. Bull. 116. n.s. 1929.
8. LOCHHEAD, A. G. and MCMASTER, N. B. Sci. Agr. 11: 351-360. 1931.

EPIDEMICS AMONG SLEDGE DOGS IN THE CANADIAN ARCTIC AND THEIR RELATION TO DISEASE IN THE ARCTIC FOX[1]

By Charles Elton[2]

Abstract

An enquiry was made by the Hudson's Bay Company into the origin and spread of a serious disease resembling epidemic encephalitis of silver foxes which periodically destroys large numbers of sledge dogs in the arctic and subarctic regions of Canada. A similar disease occurs in the arctic fox, and is associated with an important four-year cycle in the numbers of the fox, which may thus form a permanent reservoir for the disease organism, or organisms. General forecasting of this fox cycle is possible, and is dependent on knowledge of the lemming cycle in the arctic, and associated climatic phenomena.

Introduction

During the course of a systematic enquiry into the fluctuations in numbers occurring among Canadian wild animals, carried out on behalf of the Hudson's Bay Company during the last five years, the author became aware of the existence of considerable epidemics among the sledge dogs of the Canadian Arctic, in particular, in the region of Baffin Island, Hudson Strait, and Hudson Bay. At the same time a certain amount of information was collected concerning a very similar disease which breaks out periodically among arctic foxes in the North; the existence of the latter was first pointed out to the writer by Mr. L. Romanet, through Professor William Rowan, and later in personal conversation.

The Hudson Bay Company sent questionnaires to certain of their posts in the North, in order to find out how much was known about the sledge dog and fox diseases, and the Company have allowed the writer to place on record here the results of the enquiry, together with some other research carried out through their organization. The author is indebted to Mr. Charles V. Sale (the late Governor), the Committee of the Company, and to Chief Factor C. H. French, the late Fur Trade Commissioner in Canada, for affording every possible facility during the course of this work.

Investigation revealed the fact that epidemics among sledge dogs are often on a disastrous scale (thus 90% of the dogs at Stupart's Bay post were killed during one visitation), and furthermore that the disease had broken out periodically for a number of years past. There is also some evidence that it has been becoming more frequent in recent years. The great importance economically, both for the white population and for the natives, of this periodic destruction of their chief means of transport in winter needs no stressing; but a study of the whole problem also has a direct bearing upon the fur trade itself, since the white fox epidemics form an important part of the violent cycle in fur production which links the white people and the natives into a common economic interest. Both aspects of the question may also have more serious

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relations still to the welfare of the Eskimos, since lack of winter transport or of fur often causes them great hardship.

It would be preferable to present the results of this research in a finished state, but it is apparent that at least several years of co-operative effort on the part of Government, commercial, and scientific bodies, will be required before the exact nature of these epidemics can be ascertained and effective control obtained. There is another aspect too: recent research in Minnesota University carried out by Dr. R. G. Green and others (6, 7, 8, 9), has brought to light a wide-spread disease, known as fox encephalitis, which may at times attack and kill a large percentage of the animals on silver fox farms. This disease resembles in many ways that which occurs in the North among dogs and foxes, and may prove to be the same. It would therefore seem important to bring together the results of these two lines of research at the earliest possible moment. Hence it was thought desirable to present at once the preliminary results of the Hudson's Bay Company's enquiry, in order to focus attention on the problem. The writer is greatly indebted to Dr. S. Hadwen for his constant advice and interest during this investigation, and to Dr. R. G. Green for some valuable suggestions.

Acknowledgment is also made of the value of the observations supplied by various officials in the Hudson's Bay Company and other persons, in particular the help given by Mr. George Binney, who made certain special enquiries about arctic fox cycles, when he was travelling round Hudson Bay, and by Mr. C. S. Townsend, late Development Manager of the Company. Their co-operation and help have made it possible to write this paper: wherever names are known the information is acknowledged in the text.

Epidemics Among Sledge Dogs

Questionnaires were framed as follows and sent out to a number of the Company's fur posts in the North:

"A fatal epidemic disease has become common among sledge-dogs in the North, and is believed to depend in some way upon similar epidemics among white foxes. The disease is shown by strange behavior on the part of the dogs, which foam at the mouth and run about in a peculiar way, finally becoming weaker and weaker until they die. It is extremely important that the nature of this epidemic should be discovered, in order that its spread may be checked. You are asked to supply any information you possess in answer to the questions given below:

"1. Has any epidemic of this sort occurred among dogs at your Post? When did it first occur?

"2. What were the symptoms of the disease?

"3. In what month of the year did it occur?

"4. Have you ever observed any epidemic disease among *White Foxes?*

"5. In what month and year did you observe it?

"6. What were the symptoms of the disease?

"7. Have you ever observed any connection between the disease in the *dogs* and that in the *White Foxes?*"

The questionnaire was based chiefly on descriptions of the disease given to the writer by Mr. W. O. Douglas who had for some years been manager of the post at Baker Lake. A summary of the replies received to these questions is given below, dealing in this section with the first three. In a few cases data from other sources are included. The places mentioned are shown on the map in Fig. 1.

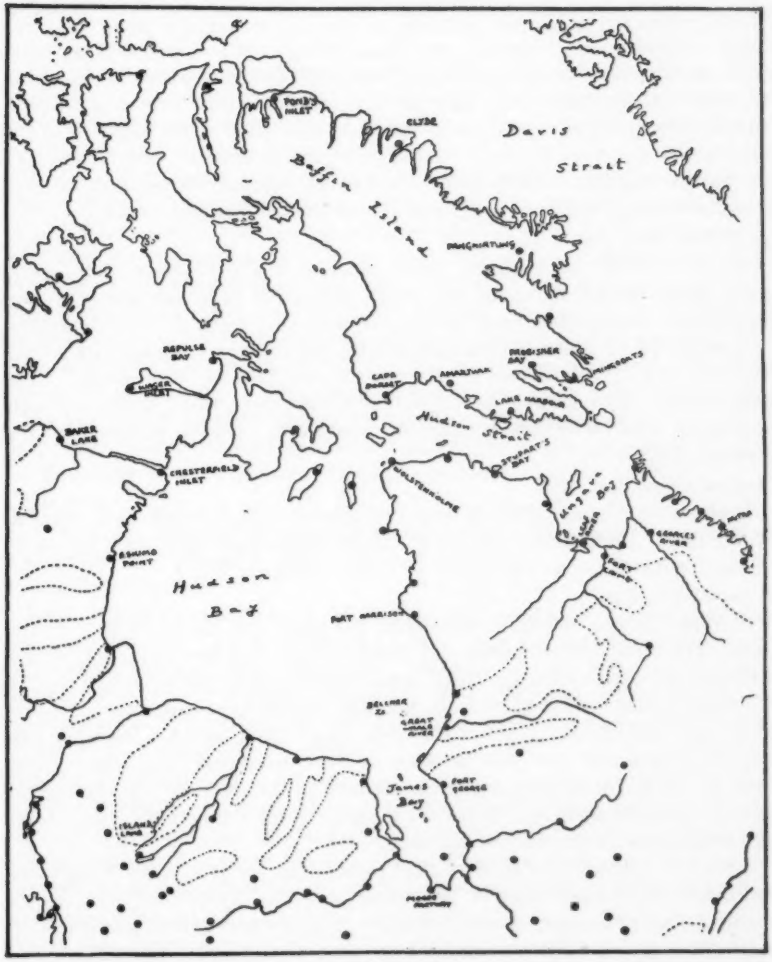

FIG. 1. *Map of Arctic Canada (based on the official map of the Hudson's Bay Company, by permission). Black dots are Hudson's Bay Company posts and outposts (except in the case of Mingookts). The map contains only names of posts mentioned in the text. Approximate tree-limit is shown by dotted line. Scale, about 270 miles to one inch.*

George's River Post

Epidemic killed many dogs about 1921 and again in 1928, March and April. Symptoms: frothing at mouth, partial paralysis and viciousness. (From Alexander Smith, summer, 1929.)

Fort Chimo Post

Epidemics twice during the last seven years. Mostly in spring and summer, but not restricted to any one month. Symptoms vary: (1) Slight foaming at mouth, apparent giddiness, snapping at any noise close by as if they were blind, vomiting, and except in rare cases, dying in the course of a few days. (2) Loss of power of hind quarters, continual snapping at all approaching, whether man or beast, and very savage, frequent recoveries, but liable to recurrence or; (3) savage at any restraint, absolutely wild, liable to attack people, run at a tree, house, or any large object that crosses their vision, or a mad desire to gallop. These three sets of symptoms may be separate, or intermingled to a varying extent. (From John Blackhall, summer, 1929.)

Leaf River Post

Winter 1928-29; an epidemic killed off most of the Eskimos' dogs, every team being reduced to one or two dogs. Broke out in March and continued until June. Symptoms: first, the dogs became very weak and bad tempered. As the disease advanced some dogs lost power over their hind quarters, others of their fore legs. All foamed at the mouth and became very savage. The sickness was not incurable as a few of the Post dogs, which were very sick, recovered after administering sulphur and lard. (From Robert Skinner, summer, 1929.)

Stupart's Bay Post

Broke out in October 1927, lasting until March 1928, killing 90% of the dogs at this Post. Symptoms: foaming at mouth, weakness of legs, dogs eventually falling down and dying. (From W. T. Watt, summer, 1929.)

Wolstenholme Post

Epidemic among the Post dogs about the middle of March 1928. Symptoms: (1) foaming at the mouth and running round in a dazed manner; (2) the dogs simply pined away; diarrhoea appeared to be the chief symptom, and in some cases small amounts of froth mingled with blood were passed. There were these two distinct types of disease. Dogs which suffered from the first only recovered and are still working. None recovered from the second. *Four or five half-breed dogs and a St. Bernard at the Post did not contract the disease.* The epidemic was at its worst amongst the young dogs, most of the recoveries being in the cases of old dogs.

A further note written in May 1930 stated that the mad type of epidemic had occurred in three dogs in the spring of 1930. Symptoms: first stage, lasting about two days, excited behavior, with tongue lolling, panting as if overheated, giving a short sharp bark or "yap" occasionally, also snapping at anything that comes near them. They then begin to "chew" and foam at the mouth, and have a peculiar glassy stare and do not seem to recognize anyone; if not tied up they will sometimes run around in a staggering manner, fall

down as if in a fit and not recover, but mostly they have to be shot. He states that any dog bitten by one with this disease usually shows the same symptoms in about eight days. (From F. Melton, summer, 1929.)

Lake Harbor Post

Epidemics in dogs a few years previous to 1926 (Post Manager's information, through Mr. G. Milling). He had noticed the hanging jaw symptom (see below).

Frobisher Bay Post

Two epidemics during the past few years. First began in November 1924, when practically all the dogs died. Symptoms: as described in the questionnaire, strange behavior, foaming at mouth, running in a peculiar way, finally becoming weaker and weaker until they died.

The second epidemic began in June 1927 and broke out again in September 1927. Symptoms slightly different: dogs became very fierce at first, and went pugnaciously among the other dogs. Small dogs in this condition easily overcame the strongest dogs, whereas before the epidemic they would cringe with their tails between their legs even at a growl from the big dogs. This stage lasted several days, after which the dogs began to foam at the mouth and go about with the mouth wide open, apparently having the jaw locked so that they could not close the mouth. The dog afterwards moved about peacefully and slowly until he died. The course of the disease lasted about three weeks. The epidemic first appeared among the Sabellum Company's dogs at Mingetook (apparently the same as Mingookts in Frobisher Bay) in April 1927. *None of the Post mongrels were affected.* (From James Bell, summer, 1929.)

Clyde Post

Some of the older natives claim they have seen a fatal disease in dogs, but a long time ago. (From J. Smith, March 3, 1930).

Pond's Inlet Post

Last epidemic in spring 1924. "During that time I believe the disease was prevalent amongst all dogs in Baffin Land". Symptoms as in questionnaire: increasing weakness leading to death. One dog became violent and chased several Eskimos around until it was finally shot. Natives remembered severe epidemic about fifteen years ago, which killed nearly all the dogs in the country. (From F. G. Troup, May 31, 1930.)

Repulse Bay Post

"This epidemic occurs at this Post regularly each spring, also often in the fall, and this year it has attacked and destroyed a number of pups during the winter. Symptoms: green pus in eyes, nose choked with same pus, partial paralysis of hind quarters. Fatal in nine cases out of ten. (From McHardy, April 11, 1930.)

Wager Inlet Post

A few pups died September 1929 (symptoms not recorded owing to absence of manager). No epidemic since. In 1928-29 nine full-grown dogs died with symptoms similar to those recorded below for Chesterfield Post. (From the Post Manager, summer, 1930.)

Chesterfield Post

Epidemic disease during 1925-1928, mostly during August to September, usually stopping as soon as the cold weather set in. Comparatively few die in winter, mostly younger dogs. Symptoms: paralysis of hind-quarters, generally howling as if in great pain, frothing at mouth, eyes glassy with yellow matter deposited around them, noses stopped up with similar substance. (From the same Post Manager mentioned under Wager Inlet Post through Mr. Hugh Conn, the Company's General Inspector; see also under Baker Lake Post and Moose Factory Post.)

Baker Lake Post

Epidemic broke out in September 1929, lasting until end of October. Five dogs died. Symptoms: foaming at mouth, complete loss of appetite, eyes dull and diffused, partial paralysis of hind quarters, would stagger round in circles and keep falling down. Later on, a chronic discharge of pus from the nose and eyes, and total paralysis of the hind quarters. This usually continued for about a week when they finally died from weakness. "In the many instances I have witnessed an outbreak of this disease, I have never known a dog to attack any person or other dog."

In a personal interview with Mr. W. O. Douglas in London, the following information was obtained. He first came to Baker Lake in 1916. At this time, he stated, the sledge dogs had epidemics periodically about every fourth year, corresponding with a similar cycle in white fox epidemics. Now (1929) the dogs had it nearly every year and it had greatly decimated them; but the fox epidemics still had a longer periodicity. Symptoms of dogs: froth at mouth, get light-headed, and tendency to run in straight lines. Become very weak, not violent, and die in about three days. Nearly always fatal. Usually in late summer or fall. Mr. Douglas also described what he believed to be a different disease in the dogs. Symptoms: eyes sunk in head, cheeks swollen below eyes, and great running at the nose. This also occurs in the fall. (From W. J. Peters, May 30, 1931.)

Epidemics observed at Baker Lake in 1918-19 and 1919-20, also observed during August 1922 at Chesterfield Post. Symptoms: foaming at the mouth, fighting, biting, madness, running about. Get weak, and usually are shot. (From H. T. Ford, Post Manager, Nonala Post, May 28, 1930.)

Eskimo Point Post

Epidemic in November and October, in 1927 or 1928. Symptoms: foaming at mouth and nose, general weakness and loss of appetite, apparent paralysis of the body. (From T. C. Carmichael, May 10, 1930.)

Evidently the same epidemic referred to by Dr. Hadwen (10) who stated, "there is one northern disease which occurs periodically and which has somewhat the appearance of rabies, but from what the writer has learned about the infection, it cannot be true rabies. Whatever be the exact nature of the disease, it attacks all canines either wild or domestic." Just as the writer had finished his paper he received a letter from the Rev. Marsh of Eskimo Point, Hudson Bay, informing him that a bad outbreak of the above disease

had occurred. The symptoms he gives are as follows: 'The dogs are again suffering from this terrible scourge. It seems to me like rabies. The cheeks swell, the nose stops up, the mouth drips saliva, the animal is gradually paralyzed (from the hind quarters usually) and very soon is dead'. He adds: 'The natives are all losing dogs and you can realize, I know, how serious a proposition this is'."

Port Harrison Post

Great numbers died in spring 1928 to middle of July. (From Post Manager.)

Fort George Post

Great numbers died during winters of 1925 and 1926 before he came to the post. None observed since. Worst in the fall and spring months. Symptoms: foaming at mouth, running around in circles, gradually becoming weaker until they died. Others would commence to shiver and die almost at once, whilst others would turn blind, their eyes becoming entirely white. The latter were usually shot, as the Indians found that they would bite at any obstruction they met with and I understand that a number of children were bitten but with no dangerous results. (From R. Gordon, June 6, 1930.)

Great Whale River Post

The sickness among dogs has occurred as long ago as the oldest Eskimo can remember. Symptoms: some go blind, others go mad and bite, others have fits while some dogs stagger about with their hind quarters useless. The Eskimos shoot any dog that shows signs of the sickness to prevent the disease spreading. (From L. G. Maver, May 10, 1930.)

Moose Factory

One of the Company's Post Managers while in London informed the author that the dog disease had gone all round Hudson Bay, *e.g.*, to Moose Factory, and that it now occurs every year. *Furthermore, that it does not attack non-sledge dogs, e.g., St. Bernard.* That this disease, or something similar, has long been established in the Bay is shown by the experience of Mr. Copley Amory, who informs me that in the early part of 1898, during the winter, there was an epidemic among the sledge dogs, when the teams arrived at Moose Factory from different directions (*e.g.*, some from Great Whale River to the north). The disease was described as "distemper".

In spite of minor disagreements (no doubt due to the different local experiences of epidemics or to generalizations made from rather few observations) the accounts leave no doubt that this sledge dog disease is a definite entity, occurring over a large part of Arctic Canada. The symptoms seem to differ not only in different dogs, but in different epidemics and in different localities. An example of the last phenomenon is the record of occasional blindness at Fort George and Great Whale River, both in southern forest region of the Bay; the emphasis laid on the hanging jaw at Lake Harbor and Frobisher Bay; the curious variations in fierceness or tameness of the dogs, etc. If the disease is a form of encephalitis, as Dr. Hadwen and Dr. Green believe probable, this variability of the symptoms is not surprising, since similar variability is well known in human encephalitis, and in the silver fox encephalitis studied by

Dr. Green. The most constant features seem to be the erratic behavior, foaming at the mouth, partial paralysis and the comparatively swift death, and the great percentage mortality accompanied by great infective power.

There was a very serious dog epidemic along the north shore of the St. Lawrence during the early part of 1930. The information about this epidemic has not yet completely come in and will be reserved for later publication. It seems probable that the disease may have been the same type as that occurring further north, but intensive work is needed before this can be decided. There were also outbreaks in northern Labrador during the winter of 1930-31, for information on which the writer is indebted to Dr. Harrison E. Kennard, who accompanied Dr. Alexander Forbes' expedition to Labrador in 1931. Most of his information was obtained from the Hudson's Bay Company Post Manager, Mr. Haynes, at Hebron. During the winter, a great many dogs were killed with the disease at Cartwright, Northwest River, Rigolet, Hopedale, and Davis Inlet posts, but Okkak and Hebron escaped. Mr. R. Bain in answer to questionnaire, summer 1929, stated that there had been no dog epidemics at Okkak, or Nutak as it is now called, for the past number of years. The symptoms were apparently similar to those described in the questionnaire. It would therefore seem probable that the north shore and the Labrador epidemics were part of the same pandemic. The old records of the Moravian missions in this region (cited by Gosling) reveal the fact that such pandemics have long been a source of loss and suffering along the coasts. "In 1859 the dogs were again attacked by the distemper which periodically visits the Labrador. The cause of this mysterious disease has not been ascertained. It seems to be rather infectious than contagious, for it breaks out simultaneously all over the coast, at places very widely separated and with no communication. The dogs in Ungava Bay were afflicted at the same time as those in Hopedale. It not only attacked the dogs but the wolves, foxes, and even the caribou died in vast numbers from the disease. We seem to know very little about the various pestilences to which wild animal life is subject" (5, pp. 301-302). Again in 1868 the "loss of sense" disease attacked the dogs (5, p. 304). Further back, in the winter of 1836-37 the distress of the hard winter "was very greatly intensified by a distemper among the dogs which causes the death of about 90% of these useful animals" (5, p. 295).

We have then evidence that these pandemics have raged periodically in Labrador for at least a hundred years. It seems that similar epidemics occur in Alaska, since Dr. Hadwen states in a letter to the author: "In Alaska all the men I spoke to believed that the dog and fox disease were one and the same, and the periodicity was as you say, four years." The dog disease is known in the Athabasca-Mackenzie region also: Mr. V. W. West, then district manager of James Bay district, wrote on March 25, 1930 that "in connection with these periodical epidemics the writer has seen several such, some of them occurring in the Upper Mackenzie district and Athabasca, where the dogs are far removed from any danger of being infected by eating the carcasses of

white foxes." The fox disease has also a wide distribution which will be referred to later.

Dr. R. G. Green has suggested to the writer the possibility that the main disease (encephalitis) is complicated either by secondary infections or by the existence of parallel but different diseases such as true distemper, or the paratyphoid also found in silver foxes (8). These questions can be solved only by intensive pathological work along experimental lines.

This paper is concerned chiefly with the mode of origin of the disease or diseases. It is obvious that epidemics might arise in several ways. They may arise spontaneously in dog teams containing the disease organism in a suppressed condition, either through lowered resistance due to food shortage or some other cause. Mr. V. W. West added in his letter that "the writer has further noted that, when such epidemics occurred, they usually commenced among the Indian dogs, which are generally in a state bordering on starvation, while the post and police dogs would be the last to become infected. Furthermore, the percentage of deaths among the Indian dogs would be very much higher than the better cared for dogs of the white people. This may only point out that the undernourished dogs are more liable to get the disease in a virulent form than would be the case with the strong, well-fed dogs. On the other hand, malnutrition may be the direct cause of the disease." It will be remembered in this connection that neither half-breed nor pure non-sledge dogs (*e.g.*, St. Bernard) were attacked in the epidemics at Frobisher Bay and Wolstenholme. In the last two epidemics all dogs were obviously exposed more or less to infection, and were evidently immune for some reason. It would be important from a practical point of view to find out whether this immunity was general (*e.g.*, due to better food and care) or specific (*i.e.*, against this particular disease). It may be noted that the fact of disease in the southern posts starting first in the Indian dogs, might be explained also by the greater contact that these dogs would be likely to have with diseased foxes or other wild animals, although on the other hand the reason may actually be that suggested by Mr. West.

Even if the disease is not derived, in the first place, from the sledge dog population itself, it seems certain that it can be spread to a large extent by contact with outside teams of dogs that have had or are having the epidemic. Although there is, I believe, general agreement that this is so, it would be in practice very important to know to what degree convalescent dogs or immune dogs can act as carriers of the disease, and how long such carriers would require to be quarantined, and what kind of quarantine is necessary or possible. We have next to consider the suggestion that the disease is derived from some other animal—in this case wild animal. This idea is discussed in the sections that follow.

Epidemics in the Arctic Fox

In this section is given a summary of the answers to questions 4, 5 and 6, in the questionnaire sent out to the posts. Names of informants as in last section. Two species of fox are mentioned. First, the arctic fox (*Alopex*

lagopus) known to the fur trade as the "white fox", normally an inhabitant of the arctic regions north of the tree limit, but migrating far to the south in years of food scarcity. It has two color phases, the winter white one, and the much rarer "blue" phase. The other species is the red fox (*Vulpes fulvus* and other subspecies or possibly species) known to the trade more accurately as "colored fox", owing to the existence of several color phases including red, cross, "silver", and black. This species normally inhabits the forest zone and regions to the south.

George's River Post

No epidemic in white foxes seen by him, but one of the natives informed him that a few years ago he saw a white fox suffering from the disease. Symptoms: frothing at the mouth and apparent madness.

Fort Chimo Post

Four dead white foxes in the fall of 1922. Symptoms: similar to those of the dog disease described on p. 676. This information mostly obtained from natives.

Mr. L. Romanet, formerly manager of the Athabasca-Mackenzie district of the Hudson's Bay Company, informed the author in 1928, that he had been at Fort Chimo in the years from about 1908 to 1916, working for Revillons Frères Trading Company. According to his observations the white fox suffers from disease in the spring and summer following the disappearance of the lemmings after their periodic peak in numbers. Symptoms: dizziness and sudden attacks of rabies-like madness. The disease also attacked dogs.

Wolstenholme Post

Had heard of the fox disease from native sources, but had not been able to secure reliable data.

Lake Harbor Post

Had seen a fox epidemic here some years previous in 1926, had found dead foxes under the rocks and seen them running *e.g.*, one ran madly into his house and was killed with a stick. The jaw was hanging in the same way as that of a dog with the disease. (Lake Harbor had its periodic fox peak in 1921, and so this epidemic was probably associated with it. C.E.)

Clyde Post

Older natives remembered disease among both dogs and foxes.

Pond's Inlet Post

Had never seen this disease among foxes, "but according to the natives a severe epidemic did occur about fifteen years ago, when it killed practically all the dogs, foxes, and wolves in the country". All these species died from the same disease. This sickness seemed to be very contagious and spread very rapidly. In recent years no disease has occurred among the foxes at this post.

Repulse Bay Post

Only place he had seen live foxes was in traps, and none of these had shown any sign of disease. Is convinced it must be very rare among them, as he had never heard the natives say anything about the disease, either here or at

Wager Inlet, Baker Lake, or Chesterfield. (This disagrees with some of the accounts given below. C.E.)

Chesterfield Post

Had never seen fox disease personally, but natives stated that several of them had come across foxes, both in winter and summer, acting in a crazy way, and they described it as the same antics that their dogs go through while affected with the above-described disease.

Baker Lake Post

One instance of a diseased fox had been observed, in first week of May, 1930. "Our interpreter was carrying ice from the Lake one day when a white fox ran up and repeatedly snapped at his legs. It finally made off and shortly afterwards ran up to a team of dogs, when it was immediately killed. Apart from the fact that it was foaming at the mouth, there seemed to me to be no similarity in the symptoms shown by the fox to those of the dogs. Its eyes were bright and sparkling, the body and fur in prime condition, and from the fact of its attacking both the man and the dogs, the symptoms showed every sign of being those of rabies" (W. J. Peters).

Mr. Douglas' observations on the periodic fox epidemics have already been mentioned (p. 678). The foxes usually have it every fourth year, and in the spring (end of April or early May). They become light-headed and are easily caught or knocked on the head, but are certainly not in a starving condition.

There was an epidemic in white foxes in the winter of 1917-1918, many being found dead. Symptoms apparently similar to those of the dogs (H. T. Ford).

Fort George Post

Had not seen any disease among white foxes, but the Indians stated that they had seen colored foxes foaming at the mouth and acting just like a dog attacked by this disease. Quite a number of colored foxes were found dead by the Indians and they appeared to think that both dogs and foxes are attacked by the same disease.

Great Whale River

Epidemics have been seen in white foxes both in winter and summer. They go mad and bite at anything within reach. Dogs are often bitten by the foxes.

The author believes that most of the information from natives must be in the main reliable, since sledge dog or white fox epidemics have the same importance for the Eskimo in the North, as a big railway accident or a coal strike have for the man in the street in Canada or England, and will be remembered just as easily.

There are the widespread native accounts of epidemics among white foxes (from George's River, Fort Chimo, Wolstenholme, Clyde, Pond's Inlet and Chesterfield Inlet) and among colored foxes at Fort George. Circumstantial stories of the disease in particular cases are given by natives from George's River and Baker Lake. In addition there are the personal experiences of some of the post managers, as at Lake Harbor, Fort Chimo, Baker Lake, and Great Whale River, and the testimony of dog owners in Alaska (given by Dr. Hadwen (see p. 680). There seems no reasonable doubt, therefore, that

white foxes in the north and (to some extent, at present unknown) colored foxes in the forest belt to the south, are liable to outbreaks of epidemic disease (usually resembling encephalitis) similar to that which attacks sledge dogs.

The area covered by this evidence includes a large part of Baffin Island, the shores of Hudson Strait, Ungava Bay, and the northwest part of Hudson Bay, also posts to the south of this, on the east side of James Bay. Since white fox disease occurs also in Alaska, it seems likely that it will be found to occur right across the Western Arctic of Canada, in the intervening region. It is of great interest to note that a similar disease may occur in Kamchatka. The author is indebted to Dr. Sten Bergman of Stockholm for amplifying the information on this subject given in his book on Kamchatka. In the book he states: "Tracks of foxes and sables were very rare that winter. During the previous year there had been an epidemic among these animals, and it had reduced their numbers to an incredible extent. The foxes which used to be extremely numerous here, had now almost entirely disappeared. In every village one heard complaints of the poor hunting." (1, p. 128).

This information refers to the colored foxes, although white foxes do also occur in the northern part of the peninsula. Dr. Bergman wrote to the author: "The exact date of the winter in which sables and foxes were scarce was 1920-1921. During that winter they were extremely scarce; I never crossed any traces of sables, and only a few of foxes, and all hunters complained of the bad hunt. The previous winter 1919-20 they were so abundant that they could be caught in the villages without any difficulty, and the inhabitants told me very often that the foxes had some disease and that they could be killed very easily with a stick as they often had lost the fear of man. I remember several kamchadals telling me that they had killed foxes when dog-driving only with the stick ("ostol") used as a breaker for the sledge. They said that the foxes often during the winter 1919-20 would run straight against a dog-team, and that they died in the woods. Sables could be caught in the villages and several inhabitants told me they had caught sables among the firewood near their houses. All said they had some disease. I arrived in Kamchatka in the summer of 1920 so I could not see the abundance myself. Of the three summers I spent in Kamchatka, mice were extremely abundant in the autumn of 1921, but not in 1920 and 1922. Hares I found very numerous both the winters I spent there (1920-21 and 1921-22). But I received a letter some weeks ago from a Swedish friend in Kamchatka, and he told me that during the winter of 1926-27 hares and mice had quite disappeared and that the sable was extremely rare." The notes on fluctuations in rodents are in themselves of great interest in showing that in Eastern Asia there are fluctuations in the animal population similar to those found in Alaska and Canada among hares, mice, and other animals. They are of particular importance here in suggesting that the deaths among foxes were not due directly to starvation, since there were enough hares, (and probably mice too ?) to provide them with food.

Similar evidence about the epidemic which attacks the arctic fox in Canada is provided by the statements of Mr. V. W. West and Mr. G. R. Ray, officials of the company who have had long experience in Canada and who

informed Mr. Binney that they could recall instances in exceptionally good white fox years when they have found many dead foxes. Death they stated was not due to starvation, the foxes being plump, but to "rabies". Snapping and snarling, they run round in circles with froth exuding from their mouths and finally fall down palpitating and die. There was an instance recorded where a missionary had the heel of his boot bitten off by a white fox in this condition. It was also stated that coyotes, wolves, and Eskimo dogs likewise contract this disease.

It is important to note that wolves are said to suffer from the same epidemic as white foxes. Statements to this effect were made by several observers already noted and by Gosling, in his account of the Labrador Moravian Missions (see p. 680). It would be very important to find out how far such epidemics act as a check upon the numbers of wolves, in view of the latter's great influence upon the caribou herds and upon other wild animals, not to speak of their direct value as fur-bearers. Gosling mentions a caribou epidemic in 1859. It would be of immense importance to know if this were really caused by the same organism which attacked the dogs, wolves and foxes in that year.

It may be concluded then, that white and colored foxes are both liable to periodic epidemics, sometimes on a very large scale, over the whole of the arctic and subarctic regions between the longitudes of 60° and 160°—in fact a third of the way round the north of the world. Before considering the relation of dog epidemics to the sledge dog epidemics it is necessary to go into the question of cycles in numbers of the arctic fox, which have an important influence on the periodicity of the disease and also on the origin of it.

Cycles in Numbers of the Arctic Fox

It is well known that the arctic fox is subject to very violent fluctuations in numbers which are sufficiently regular to merit the name of cycles. Years of great abundance are followed by rapid decrease to great scarcity, after which the numbers increase once more to a peak. This cycle is reflected in the Hudson's Bay Company's London sales: the curve for the annual number of white fox skins sold between 1850 and 1914 is to be found in Hewitt's book (11). In 1924 the author published an investigation of this cycle, and pointed out that the periodicity was one of about four years (a fact already noted by Hewitt), and that since this periodicity was the same as that of the Norwegian lemming (*Lemmus lemmus*) and since Canadian arctic foxes are supposed to depend for food upon lemmings, amongst other things, it was probable that the white fox cycle of the Canadian north could be referred back to a similar cycle in numbers of lemmings. This suggestion received support from the general agreement of the actual years of lemming abundance in Norway, and those in Canada, as deduced from the fox curve. In 1925, in another paper, it was shown that more recent white fox statistics (derived from Canadian Government royalty returns) carried the agreement up to 1922. Since that time, investigations carried through the Hudson's Bay Company and elsewhere have thrown a good deal more light on the problem. This work will be reviewed below in so far as it bears upon the periodicity of white fox epidemics: it opens

up a large number of other problems which cannot be dealt with here, and will be reserved for publication elsewhere.

Periodicity

The curve already published (2, 3, 11) represents the catch of white foxes for the whole of the Hudson's Bay Company's area of operations; in it is included the catch in posts to the south of the barren grounds and arctic regions that form the white fox's usual habitat. In the Hudson Strait area all fox skins in the earlier period came from Ungava Bay (Fort Chimo), and it was not until 1909 onwards that the white fox trapping industry was established on an extensive scale in the north, with the creation of a large number of new posts. Fort Chimo, which was established in 1828, is therefore the only post which can supply a long series of statistics from the arctic regions where the fox breeds permanently. Wolstenholme Post was established in 1909, Lake Harbor and Chesterfield Inlet Posts in 1911, followed at intervals by some 27 other posts over the whole of the eastern Arctic. Fur statistics from Fort Chimo are available from 1868 onwards, but those for one or two years are missing. If we study the continuous record since 1881, we find that the years of periodic maximum numbers were 1882, 1887, 1890, 1893, 1897, 1901, 1905, 1909, 1913, 1917, 1921, and 1926. (These dates are not the actual years in which the returns were made, but refer to the previous year: the *biological production* of white foxes takes place in the spring and summer months, the foxes are trapped in the following winter, and returns are therefore a year later than the production. In comparing lemming abundance with fox abundance, it is obviously simpler to deal with the year of actual fox production in nature rather than the year in which their skins attain commercial interest.) It will be seen that the interval between maxima is four years in seven instances, five years and three years in two instances each; the average periodicity is therefore exactly four years. There was an undoubted maximum year in 1872; certain records are missing between 1872 and 1882, but if we assume that a total of 14 cycles occurred between 1872 and 1926, the average periodicity now works out at about 3.9. It is clear that there is some very regular influence at work producing a tendency to an almost exactly four year cycle (varying occasionally to three years or five years), and which has been going on at any rate since 1872. The scarcity of white foxes in the North in 1928 was part of this periodic cycle, which has therefore continued for nearly sixty years.

This periodic cycle in white foxes is not always absolutely synchronous over the eastern Arctic. In some years (as in 1926) practically the whole of Baffin Island, Hudson Strait, and the arctic parts of the Bay have the same maximum. In 1921 a region in the southeast half of this area (Frobisher Bay, Port Burwell, Lake Harbor, Stupart's Bay, Wolstenholme, and Port Harrison) had its peak year, while the other half of the whole area (including Pangnirtung, Amadjuak, Cape Dorset, Chesterfield Inlet, Baker Lake, and Eskimo Point) had its maximum year in 1922. Again, some posts reached a peak in 1917 (as Fort Chimo did), while others reached it in 1918. The cycle is therefore not sufficiently hard and fast to be used for anything but general forecasting

as it stands, since the peak years may be single or in couples: on the other hand, the regularity over long periods, and the regional character of the years of abundance, and the resemblance to similar cycles in Norway, open up avenues of enquiry which should lead to a real understanding of the factors controlling the cycle.

Causes of the Cycle

It is well known that the arctic fox in summer is mainly dependent for its food upon lemmings and ptarmigan. While it certainly supplements these with any other animals it can obtain (particularly on the coast, where eggs and young sea-birds, offal, etc. may be found), it seems fairly certain that over the vastly greater part of its breeding ground, lemmings and birds are the main source of food, and therefore the main controlling factor in the summer breeding of the foxes.

There are only scattered data dealing with the question of lemming cycles in Canada, and the phenomenon can be best illustrated by what is known of lemmings and white foxes in Norway. The author has shown (2, 3) that if the periodic migrations of the lemmings from the Norwegian mountains are taken as an index of periodic overpopulation due to increased numbers, it appears that there have been migrations in south or central Norway almost every four years since 1862 at any rate. The actual average periodicity between the migration years is 3.9 years. In estimating this periodicity it should be noted that two years of abundance were *assumed* to exist, although no records of migrations exist. The justification for this method is proved by examination of the statistics for foxes (based on annual Norwegian Government bounty statistics) recently published by Johnsen (12). The fox curve includes both the skins of arctic and of red foxes: it shows a remarkable resemblance to the Canadian record. The maximum years of foxes (years of production, not of bounty payment) are very regular, and agree with the lemming records. There are peaks shown in the years for the two missing records of lemming migrations (implying that comparatively high numbers were reached but did not lead to migration). The fox period, *e g.*, for the Nordland District of Norway since 1880, averages exactly four years. In Norway, cycles also take place among wild mice and voles, generally in agreement with the lemming cycle, and they are an important additional, in some cases main, factor affecting fox numbers. The same is no doubt true of some of the southern parts of arctic Canada, where voles are an important element in the barren ground fauna. As regards ptarmigan, it is interesting to note that during part of the nineteenth century there was a regular cycle in ptarmigan numbers in Norway, agreeing with the lemming cycle (13). There is also evidence of a short ptarmigan cycle in arctic Canada, but not enough to determine whether it exactly synchronizes with that of the lemmings. It is clear that a short cycle in lemming and ptarmigan numbers in Canada is the most likely explanation of the main cycle of white foxes. – This hypothesis has been confirmed for certain localities. The writer does not propose to follow up this problem further in the present paper, as a very large amount of evidence connected with rodent cycles would have to be detailed and discussed.

Although the connection between foxes and lemmings may explain part of the cycle, there still remains the problem of the regional nature of the cycle, and the agreement of widely separated areas in the same years. The same question is raised by the Norwegian vole-lemming cycle, which synchronizes not only all over Norway but also with the four-year cycle in British voles worked out by Middleton (14, 15). It is difficult to escape the conclusion that some climatic factor is involved, *e.g.*, variation in the annual amount of snow cover in the winter, of the wetness or dryness of vegetation, of temperature, etc. Research on this phase of the problem is being actively carried on at present. It is clear, however, that although this research may seem to carry us very far from the original problem of sledge dog epidemics, in reality all these different subjects are intimately connected. If the sledge dog epidemics are connected with arctic fox disease, and the latter with the arctic fox cycle, forecasting of either will be dependent to a great extent on a knowledge of the causes of the fox cycle, which appear to lie with similar basic cycles among rodents and game-birds, both in turn dependent, in all probability, upon diseases and upon some climatic influence acting rather regularly over very wide areas of the Canadian and European Arctic. Climate may also be found to produce a direct cyclical influence on the arctic fox. To attain a complete understanding of this chain of events, we should have to determine the nature of the climatic factor and seek the causes of its variation in terrestrial or astronomical processes. The discovery of all these different processes will at the same time throw a great deal of light on fur trade problems and upon similar cycles in other countries.

Disease in Relation to the Fox Cycle

It is believed by a good many men living in the North that the disease attacks white foxes at the time when lemmings have disappeared, food is short, and the fox unable to resist disease. Exact information on this subject is unfortunately very limited. The replies to questionnaires give very little data about the exact years of fox epidemics, although the season is mentioned. Apparently diseased foxes have been found at various times of the year. One exact record (Fort Chimo, fall of 1922) fits in with the cycle, since the foxes were at their peak there in 1921, and would be decreasing during the following spring and summer. The other (Baker Lake, May, 1930) must await more information about recent movements of the fox cycle.

It is therefore not possible to give any conclusive statement about the periodicity of white fox disease; at the same time it seems highly probable in light of what has been said about the fox cycle, and of the general testimony of men who have had experience on the spot, that the disease does usually happen at the end of the winter or in the summer following the year of maximum numbers. There is an important possibility to note: in Norway the sudden decrease of the lemmings is caused by virulent epidemics (and also to an important extent by deaths on migration). These lemming epidemics have been studied bacteriologically in certain cases (literature summarized in (4)).

There is a well-known disease among human beings in the Norwegian valleys

called "lemming-fever", because it is supposed to be derived from water polluted by dead lemmings. This disease is also said to attack domestic animals. The whole problem has never, however, been adequately investigated. It is mentioned here to show that the periodic epidemics of the lemming can probably infect other animals at the same time. This opens up the possibility that the white fox disease is not only caused by the condition of the fox, but may even be derived directly from lemming epidemics. It is perhaps far-fetched to suggest that the migratory tendency of lemmings (attested in Canada by numerous observations which cannot here be summarized) is an expression of some nervous disease. In particular the tendency to move about in rather straight lines, and refusal to alter their direction, and the fact that they behave sometimes in quite a mad and savage manner (they will sit up and fight a man) also might be explained in the same way. This suggestion may however emphasize what may only be a casual coincidence in behavior due to quite different causes. At present we have no definite evidence that lemmings actually do have epidemics of infectious disease in the Canadian Arctic. The supposition is that they do, since the reduction in numbers during a single winter may be so very sudden and great.

It has been suggested to the author by several people that lemmings are never sufficiently numerous to control the arctic fox population. In this connection it is important to note two things: in the first place, that Norwegian lemmings are mainly nocturnal, and unless they are migrating, do not show themselves very much in the day. The great difference between a migrating lemming in Norway, and the individuals of the same species when "at home", was noted by the writer in Norway last summer. It is possible to search for lemmings in the day and find none, even when they are present in fair numbers. The second point is that the numbers of arctic foxes are probably exaggerated, since they tend to be focussed at certain points in winter, especially when they are migrating.

Migration

The arctic fox undertakes tremendous migrations, chiefly in the winter and spring. After a year of abundance in the North, it is common for a "run" of arctic foxes to be met with in the posts further south, in the margin of the forest zone, and actually also right down into the heart of this region. The extent and duration of these migrations south vary considerably. They are important for the present discussion, in so far as diseased white foxes are liable to infect dogs and wild animals south of their usual range.

Epidemics Among Red Foxes

While the white or arctic fox is normally an inhabitant of the northern regions, the forest belt is the normal home of the colored fox or red fox (including a group of species each containing the varietal phases of red, cross, silver, black, etc.). On examining the replies to several hundred questionnaires from the forest belts of Canada, extremely few records have been found of epidemics among colored foxes, although the period of investigation happened to cover the decrease of foxes following the periodic decrease of rabbits on their ten-

year cycle. This may be due partly to lack of observation, but cannot be due entirely to that since a good many epidemics among rabbits, mice, muskrats, etc. were mentioned in the answers. We may probably conclude that natural epidemics in colored foxes are far less common than they are in the arctic fox. One of the records of colored fox epidemics is accounted for by the theory that they had caught the disease of the white fox. Thus a reply from Great Whale River Post stated that "the Eskimos at the Belcher Islands report a number of white and colored foxes were found dead last summer (1927)." Two others cannot apparently be accounted for in this way. A reply from Island Lake Post, N.E. Manitoba, (G. C. M. Collin), stated that "I have talked with an Indian who states he has come across carcasses of dead foxes. He states they were in a very emaciated condition; it must be sickness as rabbits were plentiful in this locality. This was during the winter of 1929-30." Another report from Fort St. James, British Columbia, stated that there was an epidemic among colored foxes and prairie wolves between October 1927 and January 1928. Finally, the experience of silver fox farmers in Minnesota proves that encephalitis and other diseases are endemic among the silver foxes brought into captivity, unless of course they are derived from dogs.

Relation of Dog and Fox Epidemics

In those instances where both fox and dog epidemics have been observed in the same locality there is usually agreement on the part of observers that the two diseases are identical. Mr. Blackhall (Fort Chimo) stated, "It is the firm belief of the natives that it is the same sickness which attacks both dogs and white foxes. Further they believe that a close connection exists in the scarcity or abundance of lemmings in the district. When lemmings have reached their peak, foxes soon begin to decrease, and also dogs become more or less sick, showing the symptoms as described." Mr. Romanet had arrived at the same conclusion. Mr. Ford, speaking of the winter epidemic at Baker Lake in 1918-19, stated that "it is probable that the dogs were infected through eating dead foxes". Mr. Maver (Great Whale River) reported that "Eskimos state that when mad foxes bite the dogs, the latter at once are attacked with the disease and the native is positive that the sickness among the dogs originated in the white fox".

A simple comparison of the dates of the sledge dogs epidemics already recorded and those of the years of fox abundance (assumed here to be indicators of the probability of fox epidemics) shows no marked connection between the two. But, owing to the fact that teams of dogs may spread the disease from one post to another, it is not expected that any such simple relation would be found. We should expect that periodically there might be outbreaks of disease derived from foxes, which would lead to a succession of dog epidemics at intervals, probably trailing on until the next fox year. There is also the strong likelihood that recovered foxes may continue to act as carriers of the disease, thus carrying on the epidemics, as with silver foxes on farms (6). It is also clear that theoretically the fox disease might be derived from the dogs. This seems very unlikely. Or the two may be independent. This also seems unlikely;

unless the dogs depend to an important extent on wild rodent food in summer, and become infected by it. Mr. Douglas makes the important observation that the disease was formerly periodic (four years) at Baker Lake, but now occurs every year in the dogs. This indicates that in some places it has become endemic among the dogs themselves. In other areas however (as at Fort Chimo) the disease certainly does not occur every year. It is interesting to note the following statement from the manager of that post: "We are informed by our interpreter, Thomas Gordon, who has been a servant of the Company for over forty years, that it has always been an unusual thing to rear successfully young dogs on the Post, and can give no satisfactory explanation how this should be, beyond saying that he himself also experienced the same." It is suggested that *the wrong food* is the reason. A population of dogs not recruited through puppies would have different reactions to disease than one into which susceptible young dogs were constantly introduced. There appears to be a strong and growing body of evidence pointing to the disease being endemic and breaking out annually at posts on the northwest corner of Hudson Bay, but being periodic at other parts.

Summary

1. Disastrous epidemics occur periodically among the sledge dogs of the Canadian Arctic. The results of an enquiry conducted by the Hudson's Bay Company among their Posts in Baffin Island, Hudson Strait, and Hudson Bay, are recorded and discussed.

2. The commonest disease attacking the dogs resembles the "fox encephalitis" found on silver fox farms in the United States. There may be other diseases associated with this one.

3. Half-breed and non-sledge dogs are partially or completely immune to the disease.

4. A similar and probably identical disease occurs among arctic (white) foxes in the North, and appears to be associated with the periodic cycle in numbers of the foxes. Occasional records have also been made of disease in colored (red, cross, silver) foxes in the forest region of Canada, and these are possibly derived in some cases from the arctic fox. Wolves and caribou are also said to be attacked by the disease.

5. An intensive pathological study of the disease, both in dogs and in wild foxes, is required, and would probably lead to the discovery of some means of immunizing sledge dogs against the disease.

6. General forecasting of the fox epidemics is now possible. The development of this aspect of the subject depends upon a study of the rodent cycles which probably control the fox numbers, of the diseases of lemmings, and of the climatic factors which bring about simultaneous abundance and scarcity of lemmings and foxes over large areas of the Arctic.

7. If the dog disease is ultimately derived from the arctic fox, the large-scale migrations of the latter into regions south of their normal range assume a great importance, since migration appears to take place at the same time as outbreaks of disease in the foxes.

8. There is some evidence that the disease is establishing itself permanently in the dog population, *e.g.*, on the northwest shore of the Hudson Bay. In this connection a careful study of the age distribution of the disease in dogs, and of the occurrence of healthy carriers among dogs and foxes, is desirable.

9. The whole problem of sledge-dog epidemics has an extremely important bearing upon the economic life and welfare of the natives, and through them upon the prosperity of the white population in the North and elsewhere.

References

1. BERGMAN, S. Through Kamchatka by dog-sled and skis. Lippincott. 1927.
2. ELTON, C. Periodic fluctuations in the numbers of animals: their causes and effects. Brit. J. Exptl. Biol. 2: 119-163. 1924.
3. ELTON, C. Plague and the regulation of numbers in wild mammals. J. Hyg. 24: 138-163. 1925.
4. ELTON, C. The study of epidemic disease among wild animals. J. Hyg. (In press). 1931.
5. GOSLING, W. G. Labrador, its discovery, exploration and development. Toronto.
6. GREEN, R. G. Epizootic encephalitis of foxes. II. General considerations of fur-range epizootics. Am. J. Hyg. 13: 201-223. 1931.
7. GREEN, R. G. and DEWEY, E. T. Fox encephalitis and canine distemper. Proc. Soc. Exptl. Biol. Med. 27: 129-130. 1929.
8. GREEN, R. G. and SHILLINGER, J. E. Results of research on diseases of fur-bearing animals in captivity. J. Am. Vet. Med. Assen. 74: 277-282. 1929.
9. GREEN, R. G., ZIEGLER, N. R., GREEN, B. B. and DEWEY, E. T. Epizootic fox encephalitis. I. General description. Am. J. Hyg. 12: 109-129. 1930.
10. HADWEN, S. The sled dogs of the north country. Saskatoon Daily Star, March 10, 1928.
11. HEWITT, C. G. The conservation of the wild life of Canada. Scribners. 1921.
12. JOHNSEN, S. Rovdyr- og rovfuglstatistikken i Norge. Bergens Museums Aarbok. 1-118. 1929.
13. KLOSTER, R. Veksling i rypebestanden. Norske Jaeger og Fisker Forenings Tidsskrift. 50: 317-332. 1921.
14. MIDDLETON, A. D. Cycles in the numbers of British voles (Microtus) J. Ecol. 18: 156-165. 1930.
15. MIDDLETON, A. D. A further contribution to the study of cycles in British voles (Microtus) J. Ecol. 19: 190-199. 1931.

THE CONSTANCY OF REPEATED AGGLUTINATION TESTS IN THE DIAGNOSIS OF PULLORUM DISEASE[1]

By Jacob Biely[2]

Abstract

A high degree of consistency was secured in retests of five groups of birds from various sources and tested from 2 to 22 times. The results of repeated agglutination tests were, except in a few cases, confirmed by the macroscopic appearance of the ovary and by bacteriological examination.

Data show that positive reactors consistently react positive to the test, and that they seldom recover from pullorum infection. This is especially true of birds that have completed the first laying year. With these very little variation can be expected in retests at short intervals. In the case of pullets that are just starting to lay, a small percentage of reactors may throw off the infection and subsequently react negatively.

Non-reactors from an infected flock when left in contact with reactors in presence or absence of males, may in later tests react positively. This is generally due to infection through contact with infected birds, contaminated droppings, feed, water or litter. These non-reactors, if kept isolated from reactors, as a rule remain negative. When such birds become reactors at subsequent tests, the possibility of recent infection taking place just before or after the first test is not excluded. Hence, non-reactors from infected flocks should be retested at short intervals.

Suspicious reactors as a rule do not show marked variations in titre from month to month. It is impossible to predict whether a suspicious reactor will in course of time become a distinctly positive or negative reactor. With these birds therefore diagnosis must be done with care and the general condition of the flock taken into consideration. In an eradication program the bird has to be sacrificed unless it is very valuable, in which case several retests would need to be conducted before a final diagnosis is made.

Male birds do not as a rule react in as high dilutions as females, consequently fluctuations in reaction from test to test are not uncommon. Therefore, particular care must be exercised in diagnosis in the case of male birds.

Introduction

The agglutination test for pullorum disease introduced by Jones (18) in 1913 has been widely applied to commercial breeding flocks as a measure of control. Until recently it was generally believed that annual testing of flocks, and the elimination of reactors discovered at each test, would eventually result in the eradication of the disease. However, investigations during the past few years point towards the necessity of retesting flocks at short intervals (6-8 weeks), in order to ensure more rapid and certain eradication.

In a series of papers published since 1927, Beach, of Wisconsin, and his associates (1, 2, 33, 34, 35) drew attention to the variability of repeated agglutination tests and the consequent difficulties encountered in eradicating pullorum carriers from an infected flock. A similar report has been simultaneously published by Beach of California (3). The results obtained by Kernkamp (20) agree with those of the two previously mentioned investigators. Quite recently Lerche (23, 24) has reported that there was considerable disagreement between repeated agglutination tests and autopsies of non-reactors and reactors.

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Failure of reactors to react to one or more tests and fluctuations in the titres of reactors have been reported by Ericksen (13), Gwatkin (15), Doyle (9), Rice (28), Fitch and Lubbehusen (14), Newsom *et al* (25), Dearstyne *et al* (8), Bushnell and Brandly (7), and Tittsler *et al* (31).

Remarkably uniform results in repeated agglutination tests have been reported by Kaupp and Dearstyne (19), Edwards and Hull (12), Runnells (29), Rettger, McAlpine and Warner (27), Sawyer and Hamilton (30), and Biely, Sawyer, Hamilton, Johnson and Dickinson (5). The results of the repeated agglutination tests reported by the last group were in close agreement with the macroscopic appearance and bacteriological examination of the ovaries of reactors and non-reactors.

In the course of our investigations of pullorum disease in chicks and breeding stock, ample opportunity presented itself to determine the practical value of the agglutination test (4). In order to gain further information on the reliability and constancy of the agglutination test *per se* the present study was undertaken.

Material

The data presented in this paper are based on a study of five distinct groups of birds.

Group 1

This group, consisting of 98 White Wyandotte females (11 to 12 months old), was secured from a flock in which a heavy infection with pullorum disease was known to exist.* The birds were confined in a new building and placed in two separate pens, 12 by 16 ft. Pen 1 included 48 birds and Pen 2, 50 birds. Three cockerels were kept in each pen. The care and management of the birds remained constant throughout the experiment.

The birds were placed in the respective pens on May 1, and none, except those that died, removed until October 1. On October 1, 20 non-reactors were withdrawn from Pens 1 and 2 and divided equally into adjoining pens numbered 3 and 4, each 4 by 16 ft. The birds in Pen 3 were mated to a reacting cockerel and those in Pen 4 to a non-reacting cockerel.

Group 2

This group, which replaced the 20 non-reactors in Pens 1 and 2, was comprised of 12 birds that were known to be reactors. Ten of these were White Wyandotte hens and two Light Sussex hens, all 18 to 20 months old, obtained on November 5 from a flock to which the routine agglutination test had been applied.

Group 3

Thirteen two- to three-year old S.C. White Leghorn hens were secured from an infected flock 10 weeks after the application of the routine agglutination test by another laboratory. These birds were kept in a separate pen 4 by 16 ft. (No. 5) adjoining Pen 4.

**Information obtained through the courtesy of Dr. E. A. Bruce, Animal Pathologist, Health of Animals Branch, Agassiz, B.C.*

Group 4

This group consisted of 396 pullets of six breeds hatched during the season of 1927 from a reacting flock. These birds were hatched, raised and kept under similar conditions. During the first laying year the management and care of all the pullets was exactly the same.

Group 5

Eighteen cockerels of various breeds were obtained from several flocks. Some were mated at intervals with the females of Groups 1, 2 and 3.

The birds in each group were taken at random, shortly before or after the application of the first agglutination test, and thus were not specially selected individuals. Each group, however, represented a distinct flock, the history of which both before and after the application of the agglutination test was known.

Method of Testing

With the exception of the birds in Group 4, which were tested only three times, all were tested at monthly intervals, except as noted below. Blood from the ulnar vein was collected in a sterile serological test tube, which was kept level until the blood congealed and a firm clot was formed. As soon as the bleeding was completed the blood samples were taken to the laboratory, unpacked and placed in suitable racks at once.

As a rule the blood samples were allowed to stand from two to three hours at room temperature and subsequently placed in the ice chest overnight. If the blood samples were taken early in the morning, some of the tests were conducted the same afternoon; if taken late in the afternoon, the tests were conducted early next morning. The tests were thus conducted 5 to 24 hr. after the blood samples were drawn. With few exceptions the sera were always in excellent condition.

Technique of the Tube Agglutination Test

Preparation of antigen

(a) *Media*: 1.5% plain nutrient agar+0.5% peptone; pH 7.0-7.2. (b) *Cultures*: 4 strains of *Salmonella pullorum* originally obtained about 1919 from the Massachusetts Agricultural Experimental Station. (c) *Incubation*: 48 hr. at 37° C. (d) *Physiological salt solution*: Once-distilled water plus 0.85% of c.p. NaCl. (e) *Preservative*: 5 cc. of c.p. phenol to 1000 cc. of physiological salt solution. (f) *Antigen*: The culture growth is washed off with phenolized physiological salt solution and is known as stock antigen.

The stock antigen is diluted 1 part of antigen to about 15 parts of physiological salt solution (no phenol), adjusted to 0.5 turbidity on the McFarland nephelometer, and is then known as standardized antigen. Each lot of fresh antigen is then checked with a previous lot of standardized antigen, and both lots are checked against known positive and negative sera.

Previous to making the dilutions with the serum, 1 cc. of 1% c.p. NaOH solution is added to each 100 cc. of the standardized antigen.

Serum

Only clear sera are used. Hemolyzed samples are marked on the report sheet with the letter "H".

Dilutions

To make a 1:50 and 1:100 dilution—to 0.04 cc. and 0.02 cc. of serum respectively, 2 cc. of the standardized antigen are added.

Incubation

The dilutions are incubated for 24 hr. at 37° C. and held for 24 hr. at room temperature.

Recording of reactions

Symbols used are as follows: +++, ++ = positive reactions; ++? = suspicious reactions; −+ = negative reactions.

Diagnosis

A reactor is a bird whose serum agglutinates *Salmonella pullorum* antigen in a dilution of at least 1:50, showing either a +++ or ++ reaction (complete or partial agglutination). A questionable ++ reaction in a dilution of 1:50 or a +++ reaction in a dilution of 1:25 is considered as indicating a suspicious or doubtful reactor.

Rapid Method

Antigen

Antigen is prepared in a manner similar to the tube agglutination antigen except that a solution containing 12% NaCl is used and the antigen is standardized to 50 times 0.75 turbidity on the McFarland nephelometer. Each lot of fresh antigen is checked with an old lot of antigen and tested against known positive and negative sera.

Serum

As for tube antigen.

Dilutions

Serum (0.04 and 0.02 cc.) is deposited on a glass plate ruled in 1½-in. squares. Antigen (0.2 cc.) is added to the respective quantities of serum and stirred.

Incubation

Tests are incubated for five minutes at 37° C. First reading of tests is made at the completion of incubation period; second reading, two to three minutes after exposure to room temperature after incubation.

Recording of reactions

Same as for tube method.

Diagnosis

In making a diagnosis consideration is given to degree of reaction and the time required for the reaction to occur.

In conducting the experimental tests five dilutions were employed; *viz.*, 1:10, 1:25, 1:50, 1:100 and 1:200. After October 1 the tube agglutination method was checked by the rapid method. Previous experience with the

rapid method showed that it was equally as reliable as the tube method in detecting carriers.

The method of conducting the agglutination tests remained constant throughout the course of the experiment. The tests were conducted at approximately 30-day intervals, except in June 1927 and July 1928, when the tests were made at 14-day intervals. No test was made in February 1928. Thus from May 1, 1927, to January 25, 1929, inclusive, 22 tests were conducted.

Post-mortem Examination

All surviving birds of Groups 1, 2, 3 and 5 were killed at the end of the experiment and post-mortems conducted. Birds that died in the course of the experiment were usually examined on the same day. Note was made of the general condition of the viscera, whether the birds were in lay, the macroscopical appearance of the ovary, and the presence or absence of *S. pullorum*. In making a bacteriological examination of the ovary at least five or six abnormal ova were cultured on agar slants. In the case of birds not in lay, the dormant ovary was crushed in a sterile crucible and smears made on slant agar. The ovaries of some birds were crushed and sown completely into broth, and plated or transferred on agar slants.

These cultures were stained by Gram's method and if Gram negative were plated. Several colonies were picked from each plate and were subsequently planted in dextrose, maltose, mannitol, lactose, sucrose and xylose broth. Brom Thymol Blue was used as an indicator and 1% of each of the sugars was added. The carbohydrates were sterilized by the intermittent process for three days and checked for sterility. The majority of the cultures were checked against positive serum from natural reactors.

Experimental

The details of the monthly retests and various dilutions are too extensive to be presented in full. The data are therefore summarized in the tables. In order to facilitate further the discussion of the data, each group of birds is considered separately.

Results

Group 1

The birds were tested for 20 consecutive months and the results are shown in Table I. All surviving birds were tested 22 times, while records of 2 to 21 tests are available of those birds that died in the course of the experiment.

Post-mortem examination of 27 of the 34 birds that reacted consistently negative showed perfectly normal ovaries, while two birds contained two to three flabby ova; one had one hemorrhagic ovum; one was affected with dropsy and had several flabby ova; and one had blackish cysts attached to the ovary. Although the ovaries of these five did not appear absolutely normal, none showed the presence of typical lesions of pullorum disease. A careful bacteriological search for *S. pullorum* was negative in all cases. Two were not subjected to post-mortem examination.

TABLE I

SUMMARY OF REPEATED AGGLUTINATION TESTS OF 98 WHITE WYANDOTTE FEMALES CONDUCTED AT 30-DAY INTERVALS

No. of times tested	No. of birds consistently negative	No. of birds consistently positive	No. of birds N/P*	No of birds suspicious or showing fluctuations
22	22	19	10	8
21			1	
20		1		
18		1		
16	1	5		
15	1	2		
13		2		
12	1	5		1
11				1
10	2	2	1	
7	2	2		
6	1			
3	1	1		
2	3	2		
TOTALS	34	42	12	10

**N/P = Negative at the beginning of the experiment, but positive at later tests.*

NOTE:—*Fortnightly tests were conducted in June 1927 and July 1928. No test was made in February 1928.*

For post-mortem results see preceding text.

Post-mortem examination of 41 of the birds which reacted consistently positive showed in 40 the presence of typical lesions. In some, 90% of all ova were abnormal, while in others only a few appeared diseased. It is interesting to note that 7 out of the 41 birds were laying internally, and that one had pericarditis. *S. pullorum* was isolated from 39. In the case of the two from which *S. pullorum* was not isolated, one bird laid internally, and the plates were overgrown with contaminants, while the cultures of the other appeared sterile.

Ten of the birds indicated in Column 4, Table I, were in Pen 1, and 2 in Pen 2. The change in the reaction from negative to positive occurred during the following months:— *Pen 1:* June, 1; July, 1; August, 3; September, 3; and January, two birds. *Pen 2:* October, 1; and April, one bird.

Since there were originally 25 and 23 non-reactors in Pens 1 and 2 respectively it is obvious that there was a difference in the rate of spread of infection. It should be noted that one of the two reactors (Pen 2) which showed a change in reaction from negative to positive, did not actually react positively until six months after she had been removed, with 19 other non-reactors, to Pens 3 and 4 as noted above. Since these 20 non-reactors were kept in the same building as the reactors, infection of the bird after removal from direct contact with reactors is not excluded. No spread of infection was observed in Pen 3, in which the non-reactors were mated to a strongly reacting cockerel (from October 1927 to February 1929).

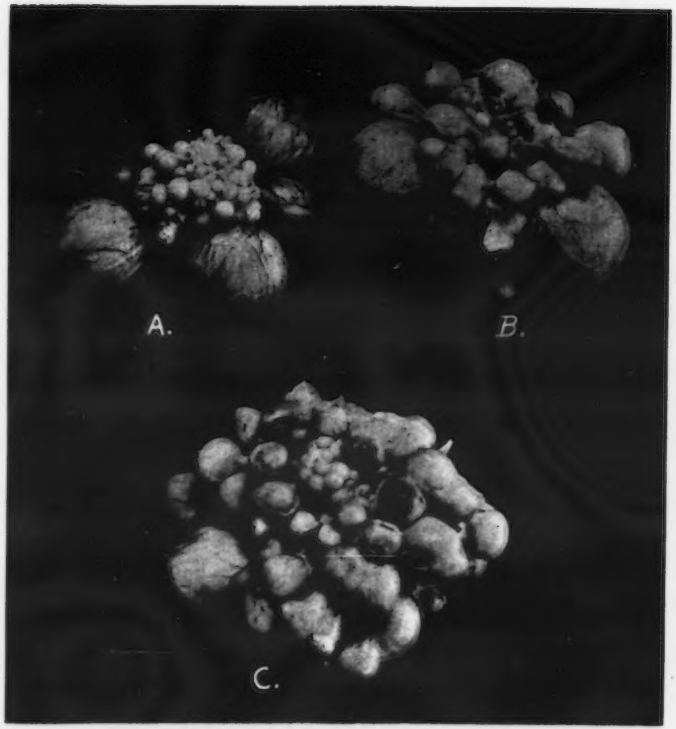

FIG. 1. *Normal and S. pullorum infected ovaries. A.—normal. B, C,—typical S. pullorum diseased ova.*

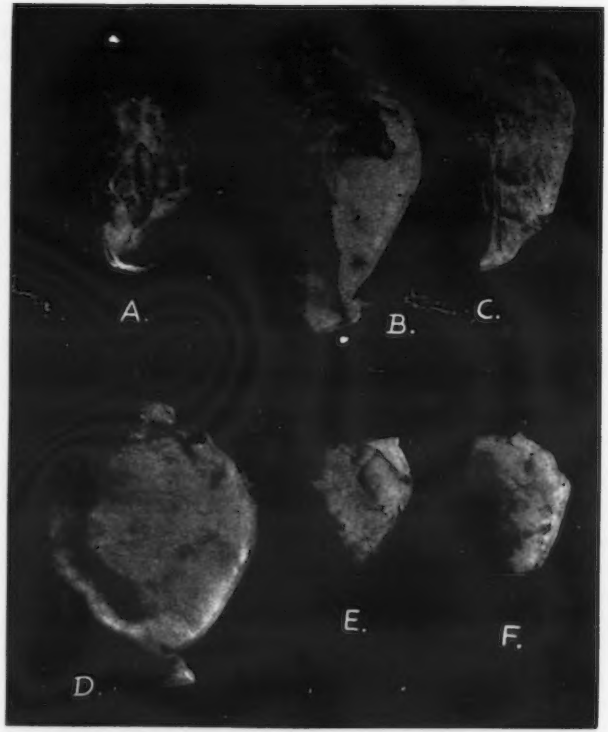

FIG. 2. *Normal and S. pullorum infected hearts. A, B, C, D—purulent pericarditis. E, F—normal hearts.*

PLATE I

TABLE II

RETESTS OF BIRDS SHOWING SUSPICIOUS OR FLUCTUATING REACTIONS TO THE AGGLUTINATION TEST

Number of bird	May	June	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Mar.	Apr.	May	June	July	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
145	+/25	+/25	+/25	++/25	++/10	++/25	+/10	+++/25	+++/25	+/25	−	−	+/25	−	+/10	−	−	−	−	−	−	−
159	++/25	++/10	++/50	++/25	++/25	++/50	++/25	++/25	+++/25	++/50	+/10	+/10	−	−	+/50	++/10	−	−	−	−	++/10	−
189	++/25	+/25	+++/25	++/25	++/25	++?/50	+/10	+/25	+++/25	+/50	0	+/25	−	−	−	++/50	−	−	−	−	−	−
191	++/50	++/25	++?/50	++/25	+/25	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
206	++/25	+++/25	+++/25	++/50	++/50	+++/50	+++/25	++/50	+++	++/25	0	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
255	+++/25	+++/10	+++/25	++/25	+++/25	−	−	−	+++/25	+/25	−	−	−	−	+++/10	++/10	−	−	−	−	−	+++/10
258	−	−	+++/25	+/25	−	+/25	−	−	−	+/25	+/10	+/25	+/10	++/10	++/10	+/10	+/25	++/10	+/10	++/10	−	−
297	+++	+++	+++	+++	+++	+++	+++/50	+++/50	+++/50	++/50	+++	++/50	++/50	++/50	++/100	++/50	++/50	+++/25	++/25	++/25	+++/10	+/25
173	−	−	−	++/100	++/50	+/25	++/25	−	−	−	−	Dead										
267	++?/50	++/25	+++/25	+++/50	+++	+++	+++	++/50	+++/25	++/50	++/100	++/100	Dead									

NOTE:—*Numerals indicate highest dilution at which reaction took place; where no numerals appear under a +++ sign, a positive reaction in a dilution of* $\frac{1}{100}$ *or* $\frac{1}{100}$ *and* $\frac{1}{200}$ *is indicated.*

− = Negative reaction; + = Negative reaction; ++? = Suspicious reaction; ++ = Positive reaction; +++ = Positive reaction; 0 = Not tested.

The evidence of progressive infection in this group of birds confirms the earlier work of Rettger *et al* (26), that pullorum disease spreads amongst mature fowls. In view of Kerr's (22) findings that carrier birds liberate *S. pullorum* in the faeces, and the recent work of Edwards and Hull (11), Brunett (6), Kernkamp (21), and Warrack and Dalling (32), that pullorum disease spreads amongst mature fowls, it is safe to conclude that the 12 originally non-reactors became actually infected in the course of the experiment.

Post-mortem examination of the same 12 birds revealed typical lesions of pullorum disease in all, while two were internal layers; *S. pullorum* was isolated from 10. In one case *S. pullorum* could not be isolated, while another bird was not examined bacteriologically, because of internal laying and advanced peritonitis.

Table II shows the degree of reaction in the highest dilution that occurred at each monthly retest. It will be seen that 5 out of the 10 birds never showed a distinct positive reaction in a 1:50 dilution. In routine testing these five birds would ordinarily have been considered negative, with the exception of one bird (No. 159), which in several retests gave a ++ reaction in a dilution of 1:50. On post-mortem examination the ovaries of these birds appeared normal, except that the ovary of one showed a flabby ovum, while the ovary of another showed one hemorrhagic and one flabby ovum. *S. pullorum* could not be isolated from any of these birds.

Three of the ten birds which showed, at the beginning of the experiment, a suspicious reaction in a dilution of 1:25 or 1:50, subsequently reacted consistently positive. *S. pullorum* was isolated from only two of the three birds, although typical lesions of pullorum disease were present in all of them.

One bird (No. 297) which gave a distinct +++ reaction in a dilution of 1:100 at the beginning of the experiment, showed a gradual decrease in the reaction and finally reacted negative. On post-mortem examination the ovary appeared normal; a culture which has not been identified as *S. pullorum* was isolated.

One bird (No. 173) showed inconsistent reactions to the agglutination test, having reacted respectively three times negative, twice positive, twice suspicious and four times negative. On bacteriological examination this bird was a non-reactor and the ovary appeared normal.

Considering the large percentage of reactors present in this group, the long contact period that existed between the reactors and non-reactors, and the consequent chances for infection, remarkably uniform results were secured in the retests.

Group 2

The 12 birds constituting this group were tested from 2 to 16 times. Four birds were tested 16 times, one 12, two 6, two 5, two 4 and one 3 times. These birds reacted consistently positive at all retests. A clear-cut reaction was obtained in the 1:100 dilution, and only a very few failed to react in the 1:200 dilution. There was also perfect agreement between the tests and the results of the post-mortem examination and cultural observations.

The flock from which these birds were secured was retested six weeks after removal of the reactors, at which time one more reactor was identified. Subsequent retest of the non-reactors of this flock and its pullet progeny showed that pullorum disease was eradicated from this flock, after the application of two tests.

Group 3

Thirteen White Leghorns in this group were under observation for 22 months and were tested 24 times in the author's laboratory and once in another laboratory. At the beginning of the experiment, *i.e.*, two and one-half months after the application of the routine test, a retest of the birds (first test of the experimental period) showed that one bird was negative to the test. This bird reacted negatively three consecutive times. Post-mortem and bacteriological examination confirmed the results of the repeated agglutination test.

A second bird, which showed a distinct +++ reaction in a dilution of 1:50 and 1:100 when tested by the previously mentioned laboratory, showed on retest a faint ++ reaction in a dilution of 1:50. In subsequent tests this bird showed considerable fluctuation in titre, although it never showed a distinct positive reaction in a dilution of 1:50 or 1:100. At time of post-mortem the bird was highly emaciated, and the ovary was completely dormant. *S. pullorum* was not isolated from this bird.

TABLE III

SUMMARY OF RETESTS OF 396 PULLETS TESTED THREE TIMES IN THE PULLET YEAR

	1st Test		2nd Test				3rd Test							
	Number birds tested	Number reactors	Number birds tested	Number reactors	Increase in reactors	Decrease in reactors	Number birds tested	Number reactors	Increase in reactors	Decrease in reactors	Total number reactors	Negative birds died	Positive birds died	Total birds died
S.C. W. Leghorns	114	9	107	8	1	3	96	4	0	0	9	12	3	15
R. I. Reds	105	13	96	13	3	1	86	9	4	0	20	11	8	19
B. P. Rocks	91	5	87	5	0	0	84	5	0	0	5	7	0	7
W. Wyandottes	51	13	44	11	1	1	38	14	6	0	20	8	5	13
Black Orpingtons	21	8	21	10	2	0	18	9	2	0	12	0	3	3
Light Sussex	14	2	14	2	0	0	13	2	1	0	4	0	1	1
Total	396	50	369	49	7	5	335	43	13	0	70	38	20	58

The remaining birds were tested as follows:— two, 24 times; two, 21 times; one, 15 times; one, 14 times; two, 10 times; one, 3 times; one, twice; and one, once. Each bird reacted consistently positive to each of the tests. The results of the post-mortem, bacteriological and serological tests were in perfect agreement.

Except for one bird, which had been originally recorded as a suspicious reactor, but reacted positive to the third test, pullorum disease would have

been eradicated from this flock, after two routine tests, conducted at six-month intervals. The pullets from this flock were not, however, free from pullorum disease. Unfortunately, the efforts to eradicate the disease failed, due to the known introduction of infected breeders early in the breeding season.

Group 4.

Pullets (396), of six breeds, were tested three times:— (1) at the commencement of the laying year; (2) in the middle of the laying year; and (3) at the end of the laying year. The birds of this group were not subjected to a post-mortem examination. The age of the non-reactors and reactors at the first test varied from 186 to 253 days. The average egg production of the whole flock was 21%, at the time of the first test, and only 15 out of the 50 reactors had laid.

A summary of the first, second and third tests is given in Table III. It will be seen that out of 396 pullets tested the first time, 50 pullets reacted positively to the agglutination test. Between the first and second tests, 27 birds died, of which 7 were reactors and 20 non-reactors. Of the 369 birds tested the second time, 49 pullets reacted positively. Seven of these 49 birds had reacted negatively to the first test. Five birds that had reacted positively to the first test failed to react to the second test. Between the second and third tests 34 birds died; of these 13 were reactors and 21 non-reactors, so that 335 birds were available for a third test. None of the birds that reacted positively to the second test reacted negatively to the third test. However, the five birds that reacted positively to the first test and reacted negatively to the second test, again reacted negatively to the third test. With the exception of these five birds the results of retesting reactors were consistent.

The apparent spread of infection in the non-reactors may have been due to direct contact with infected birds, contaminated droppings, feed and litter, or water, since no male birds were kept in this group. There is, of course, the possibility that some of the birds that reacted negatively to the first test but positively to the second test, had actually been infected with *S. pullorum* organisms prior to the first test, but had not developed sufficient agglutinins to react at that time. This possibility is however very remote in the case of the birds that reacted positively to the third test, but negatively to the second and first tests. It appears more probable that the close contact that existed between the non-reactors and reactors during the experiment was responsible for the development of infection and subsequent appearance of agglutinins in birds that reacted positively either to the second or third test. In this connection it should be noted that Hinshaw (16, 17) has observed that, even though reactors are removed from pullet flocks after each test, *infected* birds are subsequently detected.. Presumably these birds were infected, but did not produce sufficient agglutinins to give a positive reaction. The time interval required to develop agglutinins would, on the basis of Hinshaw's results, be so short that at least the 13 birds that were negative on the second test but positive on the third, must have become infected subsequent to the first test.

At the end of the laying year all the reactors were removed. A fourth test

was conducted on the non-reactors kept for breeding purposes. No reactors were found, and the progeny from this group of birds was free from pullorum disease.

TABLE IV

REPRESENTATIVE TESTS OF MALE BIRDS

Male No.	1584						257						1446					
Dilution	R*	10	25	50	100	200	R	10	25	50	100	200	R	10	25	50	100	200
April	−	++	+	−	−	−	−	++	++	+	−	−	−	++	++	−	−	−
May	−	+++	+	−	−	−	+	+	+	++	+	−	−	+++	+++	++?	−	−
June	−	+++	+++	+	−	−	+	++	++	++	−	0	0	+++	+	−	−	0
June	−	+++	+++	++	−	−	++	+++	+++	+++	+	−	0	+++	++	−	−	−
July	−	+++	++	−	−	−	+++?	++	+++	−	−	0	−	++?	+	−	−	−
August	−	++	++	−	−	−	−	+++	++	+	−	−	−	0	0	−	−	−

Male No.	1312						1357						1					
Dilution	R*	10	25	50	100	200	R	10	25	50	100	200	R	10	25	50	100	200
April	+++	+++	+++	+++	++	−	+++	+++	+++	++	++	+	+++	+++	+++	+++	+++	−
May	+++	+++	+++	+++	++?	−	+++	+++	+++	++	++	+	+++	+++	+++	+++	+++	−
June	+++	+++	+++	+++	+	+	0	0	+++	+++	−	−	+++	+++	+++	−	−	−
June	+++	+++	+++	+++	+++	+	+++	+++	+++	+++	+++	+++	++	+++	+++	+	−	−
July	+++	+++	+++	+++	++	+	+++	0	++	++	++	+	+++	+++	+++	−	−	−
August	+++	+++	+++	+++	+++	+	+++	++	+++	++	+	+	+++	+++	+++	−	−	−

*R = *Rapid method.* 0 = *Not tested.*

Group 5

Some of the male birds considered in this group were mated with the females of Groups 1, 2 and 3, while others were kept in a separate pen for retests only. Altogether 18 males were tested for various periods of time. Four of these were consistently negative even though kept in contact with reactors from seven to twenty months. On post-mortem examination these birds appeared perfectly normal. *S. pullorum* could not be isolated from the testes or pericardial sac. The remaining 14 males were considered as either suspicious reactors or reactors at the time the experiment was started. Ten of these reacted consistently positive, and were tested from 4 to 14 times, while the 4 suspicious males were tested 8, 12, 12 and 13 times respectively.

The results of some of the individual retests are presented in Table IV. Limitation of space does not permit a complete presentation of the retests of all the birds. However, the retests shown are characteristic of the group as a whole. It will be seen that there was considerable fluctuation in the reactions of the individual males from test to test. This is particularly true of males

whose sera agglutinated *S. pullorum* antigen in low dilutions only. Doyle's (10) observation that "the degree of reaction to the agglutination test in cock birds is less marked than in hens" is confirmed by the results secured in this investigation. In view of the difficulty of demonstrating lesions of pullorum disease or isolating *S. pullorum* from male birds, the significance of low agglutination titres is doubtful. The apparent discrepancies between the tube and rapid agglutination tests, in the reactions of certain male birds, further accentuate the difficulties of interpreting the reactions of male birds. In some cases this difficulty did not occur. Thus those males that showed a distinct +++ reaction in a dilution of 1:100 or 1:200 as a rule reacted consistently positive in the dilution considered diagnostic in these studies, *i.e.*, 1:50. It should be noted that in spite of variations in diagnoses from test to test, male birds that have once reacted positively to the agglutination test seldom show a complete loss of agglutinins.

TABLE V

SUMMARY OF SEROLOGICAL, GROSS AND BACTERIOLOGICAL FINDINGS OF GROUPS 1, 2, 3 AND 5

Group	Number of birds		Pullorum lesions			*S. pullorum*		*N/E*
			P	*N*	*N/E*	*P*	*N*	
1	Negative	34		32	2		32	
	Positive	42	40	1	1	39	2	1
	N/P	12	12			10	1	1
	Suspicious	10	3	7		2	8	
2	Positive	12	12			12		
3	Negative	1		1			1	
	Suspicious	1		1			1	
	Positive	11	11			11		
5	Negative	4		4			4	
	Positive	10	3	7		5	5	
	Suspicious	4		4			4	

P = positive; N = negative; N/E = not examined.

Macroscopic lesions were found in three out of the ten reacting males and *S. pullorum* isolated from five. The males that gave a suspicious reaction appeared to be normal, and *S. pullorum* could not be isolated from either the pericardial sac or the testes.

Summary

The data presented in this paper are briefly summarized in Table V, which shows the results of serological, gross and bacteriological findings of birds in Groups 1, 2, 3 and 5. The results of Group 4 have already been shown in Table III. On this group of birds, no post-mortem examinations were conducted.

It will be seen from Tables III and V that a high degree of consistency was secured in retest of the five groups of birds obtained from various sources, and tested from two to twenty-two times. The results of the agglutination tests

were, except in a few cases, confirmed by the macroscopic appearance of the ovary and by bacteriological examination.

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References

1. Beach, B. A., Halpin, J. G. and Lampman, C. E. J. Am. Vet. Med. Assen. LXX, n.s. 23: 605-611. 1927.
2. Beach, B. A., Holmes, C. and Strange, C. R. J. Am. Vet. Med. Assen. LXXVI, n.s. 29: 557-561. 1930.
3. Beach, J. R. Hilgardia, 2: 529-544. 1927.
4. Biely, J. Sci. Agr. 9: 413-422. 1929.
5. Biely, J., Sawyer, C. E., Hamilton, C. M., Johnson, W. T. and Dickinson, E. M. J. Am. Vet. Med. Assen. LXXIX, n.s. 32: 19-36. 1931.
6. Brunett, E. L. J. Am. Vet. Med. Assen. LXXVI, n.s. 29: 667-669. 1930.
7. Bushnell, L. D. and Brandly, C. A. J. Am. Vet. Med. Assen. LXXIV, n.s. 27: 444-453. 1929.
8. Dearstyne, R. S., Kaupp, B. F. and Wilfong, H. S. N. Carolina Agr. Exptl. Sta. Bull. 36: 3-53. 1929.
9. Doyle, T. M. J. Comp. Path. Therap. 38: 266-282. 1925.
10. Doyle, T. M. The Harper Adams Utility Poultry Journal, 12: 421-423. 1927.
11. Edwards, P. R. and Hull, F. E. J. Am. Vet. Med. Assen. LXXV, n.s. 28: 333-336. 1929.
12. Edwards, P. R. and Hull, F. E. J. Am. Vet. Med. Assen. LXXV, n.s. 28: 765-768. 1929.
13. Ericksen, S. Missouri State Poultry Assen. Year Book, 31-33. 1923.
14. Fitch, B. S. and Lubbehusen, R. E. Proc. Third World's Poultry Congress, Ottawa, Canada, 356-360. 1927.
15. Gwatkin, R. Report, Ont. Vet. College, 44-64. 1925.
16. Hinshaw, W. R., Sanders, E. F. and Dunlap, G. L. Mass. Agr. Exptl. Sta. Control Ser. Bull. 43. 1928.
17. Hinshaw, W. R., Sanders, E. F. and Dunlap, G. L. Mass. Agr. Exptl. Sta. Control Ser. Bull. 48. 1929.
18. Jones, F. S. J. Med. Research, XXVII, n.s. 22: 481-495. 1913.
19. Kaupp, B. F. and Dearstyne, R. S. N. Carolina Agr. Exptl. Sta. Bull. 235: 3-16. 1928.
20. Kernkamp, H. C. H. Cornell Vet. 19: 357-367. 1929.
21. Kernkamp, H. C. H. J. Am. Vet. Med. Assen. LXXVII, n.s. 30: 280-293. 1930.
22. Kerr, W. R. J. Comp. Path. Therap. 43: 77-85. 1930.
23. Lerche, Tierarztlichen Rundschau. 36: 332-335. 1930.
24. Lerche, Tierarztlichen Rundschau. 36: 346-350. 1930.
25. Newsom, I. E., Cross, F. and Ufford, O. C. J. Am. Vet. Med. Assen. LXXII, n.s. 25: 611-617. 1928.
26. Rettger, L. F. and Stoneburn, F. H. Conn. Agr. Exptl. Sta. Storrs. Bull. 68. 1911.
27. Rettger, L. F., McAlpine, J. G. and Warner, D. E. J. Am. Vet. Med. Assen. LXXVII, n.s. 30: 47-57. 1930.
28. Rice, J. P. Nat. Poultry J., v. 216. 1924.
29. Runnells, R. A. Va. Agr. Exptl. Sta. Bull. 265: 3-27. 1929.
30. Sawyer, C. E. and Hamilton, C. M. West. Wash. Exptl. Sta. Bull. 17-W. n.s. 1-19. 1930.

31. TITTSLER, R. P., HEYWANG, B. W. and CHARLES, T. B. Penn. Agr. Exptl. Sta. Bull. 235: 3-16. 1928.
32. WARRACK, G. H. and DALLING, T. Vet. J. 87: 24-27. 1931.
33. Wisconsin Agr. Exptl. Sta. Bull. 396. Ann. Rep. of the Director, 54-57. 1927.
34. Wisconsin Agr. Exptl. Sta. Bull. 405. Ann. Rep. of the Director, 31-32. 1929.
35. Wisconsin Agr. Exptl. Sta. Bull. 410. Ann. Rep. of the Director, 69-70. 1930.